

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
8 November 2007 (08.11.2007)

PCT

(10) International Publication Number
WO 2007/127010 A2

(51) International Patent Classification: **Not classified**

(21) International Application Number:

PCT/US2007/007854

(22) International Filing Date: 29 March 2007 (29.03.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/786,837 29 March 2006 (29.03.2006) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

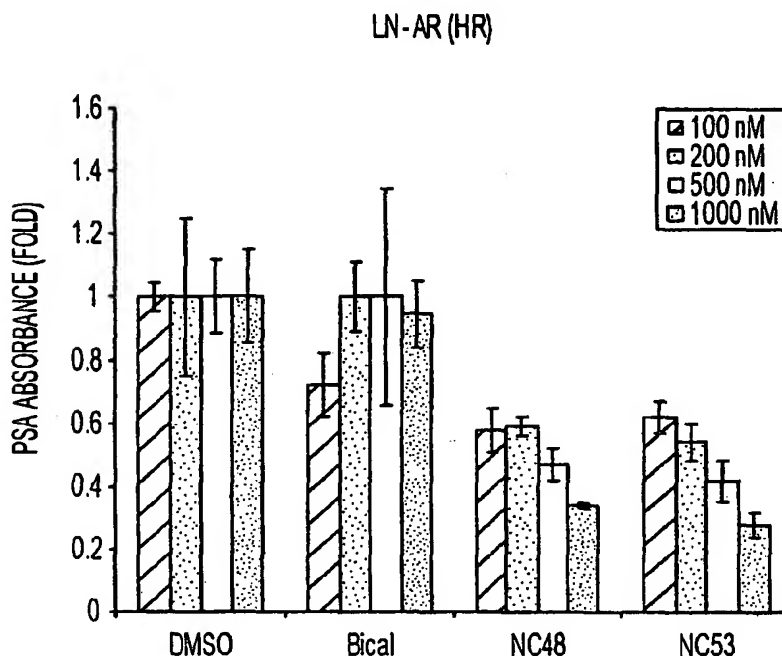
(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))

[Continued on next page]

(54) Title: DIARYLTHIOHYDANTOIN COMPOUNDS



(57) Abstract: The present invention relates to diarylthiohydantoin compounds and methods for synthesizing them and using them in the treatment of hormone refractory prostate cancer.

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- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))
- of inventorship (Rule 4.17(iv))

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Published:

- without international search report and to be republished upon receipt of that report

DIARYLTHIOHYDANTOIN COMPOUNDS

FIELD OF THE INVENTION

[0001] The present invention relates to diarylthiohydantoin compounds including diarylthiohydantoins, and methods for synthesizing them and using them in the treatment of hormone refractory prostate cancer. This application incorporates by reference PCT/US2006/011417 by the same assignee.

BACKGROUND OF THE INVENTION

[0002] Prostate cancer is the most common incidence of cancer and the second leading cause of cancer death in Western men. When the cancer is confined locally, the disease can be cured by surgery or radiation. However, 30% of such cancer relapses with distant metastatic disease and others have advanced disease at diagnoses. Advanced disease is treated by castration and/or administration of antiandrogens, the so-called androgen deprivation therapy. Castration lowers the circulating levels of androgens and reduces the activity of androgen receptor (AR). Administration of antiandrogens blocks AR function by competing away androgen binding, therefore, reducing the AR activity. Although initially effective, these treatments quickly fail and the cancer becomes hormone refractory.

[0003] Recently, overexpression of AR has been identified and validated as a cause of hormone refractory prostate cancer. See Chen, C.D., Welsbie, D.S., Tran, C., Baek, S.H., Chen, R., Vessella, R., Rosenfeld, M.G., and Sawyers, C.L., Molecular determinants of resistance to antiandrogen therapy, Nat. Med., 10: 33-39, 2004, which is hereby incorporated by reference. Overexpression of AR is sufficient to cause progression from hormone sensitive to hormone refractory prostate cancer, suggesting that better AR inhibitors than the current drugs can slow the progression of prostate cancer. It was demonstrated that AR and its ligand binding are necessary for growth of hormone refractory prostate cancer, indicating that AR is still a target for this disease. It was also demonstrated that overexpression of AR converts anti-androgens from antagonists to agonists in hormone refractory prostate cancer (an AR antagonist inhibits AR activity and an AR agonist stimulates AR activity). Data from this work explains why castration and anti-androgens fail to prevent prostate cancer progression and reveals unrecognized properties of hormone refractory prostate cancer.

[0004] Bicalutamide (brand name: Casodex) is the most commonly used anti-androgen. While it has an inhibitory effect on AR in hormone sensitive prostate cancer, it fails to suppress AR when cancer

becomes hormone refractory. Two weaknesses of current antiandrogens are blamed for the failure to prevent prostate cancer progression from the hormone sensitive stage to the hormone refractory disease and to effectively treat hormone refractory prostate cancer. One is their weak antagonistic activities and the other is their strong agonistic activities when AR is overexpressed in hormone refractory prostate cancer. Therefore, better AR inhibitors with more potent antagonistic activities and minimal agonistic activities are needed to delay disease progression and to treat the fatal hormone refractory prostate cancer.

[0005] Nonsteroidal anti-androgens, such as bicalutamide, have been preferred over steroidal compounds for prostate cancer because they are more selective and have fewer side effects. This class of compounds has been described in many patents such as U.S. Patent Number 4,097,578, U.S. Pat. No. 5,411,981, U.S. Pat. No. 5,705,654, PCT International Applications WO 97/00071 and WO 00/17163, and U.S. Published Patent Application Number 2004/0009969, all of which are hereby incorporated by reference.

[0006] U.S. Patent No. 5,434,176 includes broad claims which encompass a very large number of compounds, but synthetic routes are only presented for a small fraction of these compounds and pharmacological data are only presented for two of them, and one skilled in the art could not readily envision other specific compounds.

[0007] Because the mechanism of hormone refractory prostate cancer was not known, there was no biological system to test these compounds described in these patents for their effect on hormone refractory prostate cancer. Particularly, the ability of AR overexpression in hormone refractory prostate cancer to switch inhibitors from antagonists to agonists was not recognized. Some new properties of hormone refractory prostate cancer are reported in PCT applications US04/42221 and US05/05529, which are hereby incorporated by reference. PCT International Application US05/05529 presented a methodology for identifying androgen receptor antagonist and agonist characteristics of compounds. However, for each compound produced, the time consuming process of determining the antagonist and agonist characteristics of a compound must be determined. That is, there is no method to accurately predict characteristics relevant to treating prostate cancer from the chemical structure of a compound alone.

[0008] Some compounds have been reported to be inhibitors of the ligand binding domain (LBD) androgen receptor (AR). Several have been used as drugs to treat prostate cancer, e.g., bicalutamide (Casodex). Several binders of the AR LBD have been identified, e.g., the thiohydantoin, RU59063 and BTID. (Teutsch, G.; Goubet, F.; Battmann, T.; Bonfils, A.; Bouchoux, F.; Cerede, E.; Gofflo, D.; Gaillard-

Kelly, M.; Philibert, D. *J. Steroid Biochem. Molec. Biol.* 1994, 48, 111-119; Van Dort, M. E.; Robins, D. M.; Wayburn, B. *J. Med. Chem.* 2000, 43, 3344-3347)

5 [0009] There is a need for new thiohydantoin compounds having desirable pharmacological properties, and synthetic pathways for preparing them. Because activities are sensitive to small structural changes, one compound may be effective in treating prostate cancer, whereas a second compound may be ineffective, even if it differs from the first compound only slightly, say by the replacement of a single substituent.

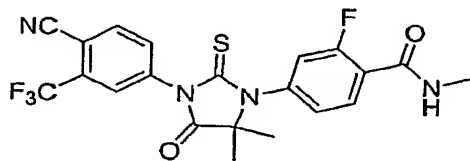
10 [0010] Identification of compounds which have high potency to antagonize the androgen activity, and which have minimal agonistic activity should overcome hormone refractory prostate cancer (HRPC) and avoid or slow down the progression of hormone sensitive prostate cancer (HSPC). Therefore, there is a need in the art for the identification of selective modulators of the androgen receptor, such as modulators which are non-steroidal, non-toxic, and tissue selective.

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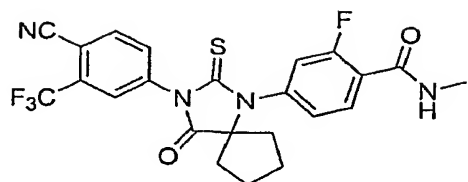
SUMMARY OF THE INVENTION

[0011] The invention provides a series of compounds having strong antagonistic activities with minimal agonistic activities against AR. These compounds inhibit the growth of hormone refractory prostate cancer.

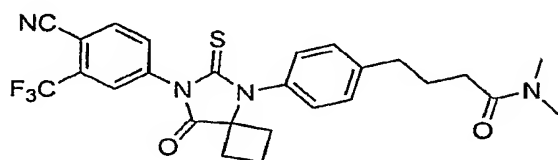
20 [0012] Particular compounds of the invention include



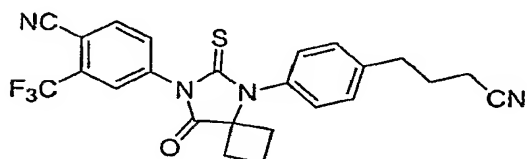
[NC54]



[NC55]



5 [NC56]



[NC57]

- 10 [0013] The invention also provides a pharmaceutical composition comprising a therapeutically effective amount of a compound according to any of the preceding compounds or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier or diluent.

- [0014] The invention encompasses a method for treating a hyperproliferative disorder comprising administering such a pharmaceutical composition to a subject in need of such treatment, thereby treating the
- 15 hyperproliferative disorder. The hyperproliferative disorder may be hormone refractory prostate cancer. The dosage may be in the range of from about 0.001 mg per kg body weight per day to about 100 mg per kg body weight per day, about 0.01 mg per kg body weight per day to about 100 mg per kg body weight per day, about 0.1 mg per kg body weight per day to about 10 mg per kg body weight per day, or about 1 mg per kg body weight per day.

- 20 [0015] The compound may be administered by intravenous injection, by injection into tissue, intraperitoneally, orally, or nasally. The composition may have a form selected from the group consisting of a solution, dispersion, suspension, powder, capsule, tablet, pill, time release capsule, time release tablet, and

time release pill.

[0016] The administered compound may be selected from the group consisting of NC54, NC55, NC56, or NC57, or a pharmaceutically acceptable salt thereof. The administered compound may be NC53 or a pharmaceutically acceptable salt thereof.

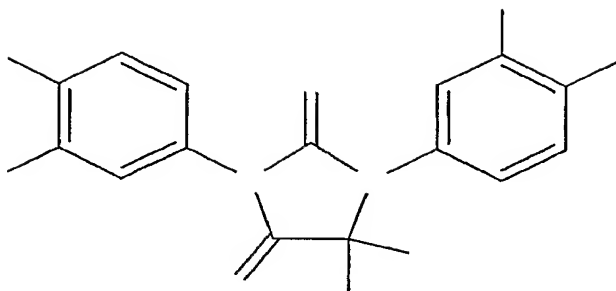
5 [0017] The invention provides a method of synthesizing NC54 comprising mixing *N*-Methyl-2-fluoro-4-(1,1-dimethyl-cyanomethyl)-aminobenzamide and 4-Isothiocyanato-2-trifluoromethylbenzonitrile in DMF and heating to form a first mixture, and processing as above.

[0018] The invention also provides a method of synthesizing NC55, comprising mixing *N*-Methyl-2-fluoro-4-(1-cyanocyclopentyl)aminobenzamide, 4-isothiocyanato-2-trifluoromethyl benzonitrile, and DMF
10 and heating under reflux to form a first mixture, and processing as above.

[0019] The invention further provides a method of synthesizing NC56, comprising mixing *N,N*-Dimethyl 4-[4-(1-cyanocyclobutylamino)phenyl]butanamide, 4-isothiocyanato-2-trifluoromethyl benzonitrile, and DMF and heating under reflux to form a first mixture; and processing as above.

[0020] The invention provides a method of synthesizing NC57, comprising mixing DMSO,
15 dichloromethane, and oxalyl chloride to form a first mixture, adding 4-(4-(7-(4-Cyano-3-(trifluoromethyl)phenyl)-8-oxo-6-thioxo-5,7-diazaspiro[3.4]octan-5-yl)phenyl)butanamide to the first mixture to form a second mixture; adding triethylamine to the second mixture to form a third mixture; warming the third mixture and quenching with aqueous NH_4Cl to form a fourth mixture; extracting an organic layer from the fourth mixture; and isolating the compound from the organic layer.

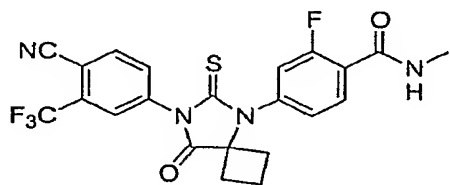
20 [0021] In an embodiment, a compound has the formula



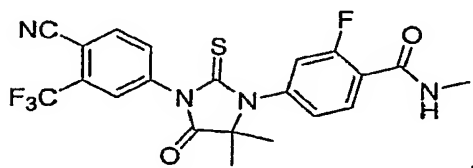
[0022] R1 and R2 are independently methyl or, together with the carbon to which they are linked, a cycloalkyl group of 4 to 5 carbon atoms, R3 is selected from the group consisting of carbamoyl, alkylcarbamoyl, carbamoylalkyl, alkylcarbamoylalkyl, cyano, and cyanoalkyl, and R4 is hydrogen or fluorine.

[0023] In an embodiment a pharmaceutical composition comprises a therapeutically effective amount of a compound according to claim 1 or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier or diluent.

10 [0024] The compound can, for example, have the formula

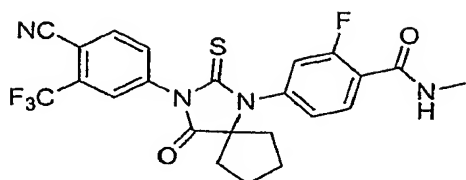


[NC53]



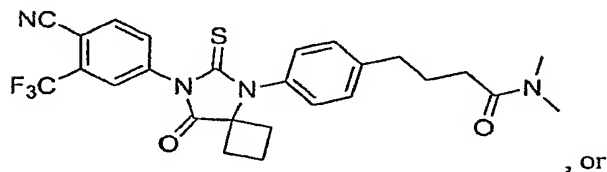
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[NC54]



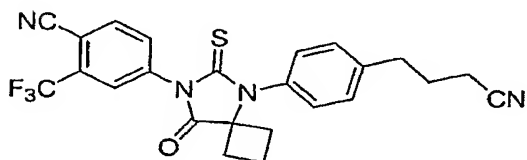
[NC55]

5



[NC56]

10



[NC57]

15 [0025] A pharmaceutical composition can comprise a therapeutically effective amount of a compound NC54 or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier or diluent. A pharmaceutical composition can comprise a therapeutically effective amount of a compound according to claim NC55 or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier or diluent.

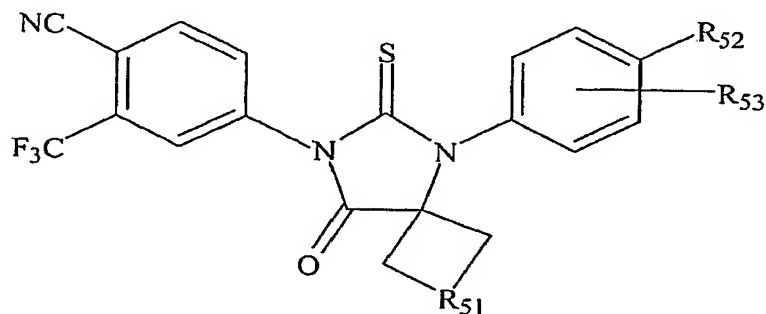
20 [0026] In an embodiment, a method for treating a hyperproliferative disorder comprises administering a pharmaceutical composition of claim 2 to a subject in need of such treatment, thereby treating the hyperproliferative disorder.

[0027] The composition can, for example, have a form selected from the group consisting of a solution, dispersion, suspension, powder, capsule, tablet, pill, time release capsule, time release tablet, and time release pill. The compound can be administered by intravenous injection, by injection into tissue, intraperitoneally, orally, or nasally. The composition can be administered at a dosage of the compound in the range of from about 0.001 mg per kg body weight per day to about 100 mg per kg body weight per day. The composition can be administered at a dosage of the compound in the range of from about 0.01 mg per kg body weight per day to about 100 mg per kg body weight per day. The composition can be administered at a dosage of the compound in the range of from about 0.1 mg per kg body weight per day to about 10 mg per kg body weight per day. The composition can be administered at a dosage of the compound of about 1 mg per kg body weight per day.

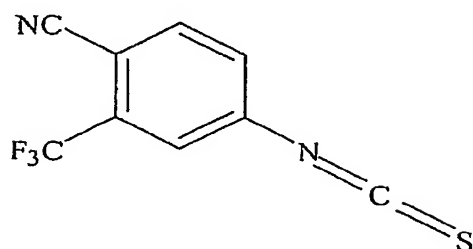
[0028] There is a method for treating prostate cancer comprising administering a pharmaceutical composition to a subject in need of such treatment, thereby treating the prostate cancer. The pharmaceutical composition can interfere with the transcription of prostate specific antigen mRNA. The pharmaceutical composition can prevent nuclear translocation of an androgen receptor protein. The pharmaceutical composition can destabilize an androgen receptor protein. The composition can be administered orally. The composition can have a form selected from the group consisting of a capsule, tablet, and pill.

[0029] In an embodiment, the compound can be NC54, NC55, NC56, NC57, a pharmaceutically acceptable salt of any of these, or combinations thereof.

[0030] A method of synthesizing a diaryl compound of formula



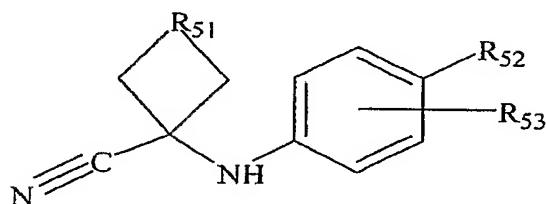
comprises mixing Compound I



Compound I

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with Compound II

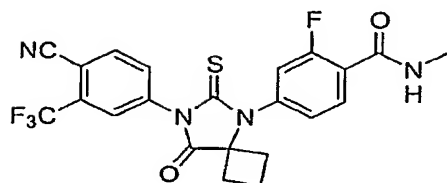


Compound II

- 10 in a first polar solvent to form a mixture, heating the mixture, adding a second polar solvent, the same as or different from the first polar solvent, and an aqueous acid to the mixture, refluxing the mixture, cooling the mixture and combining with water, and separating the diaryl compound from the mixture. R51 can include an alkyl chain of from 1 to 4 carbon atoms. R52 can be cyano, hydroxy, methylcarbamoyl, methylcarbamoyl-substituted alkyl, methylsulfoncarbamoyl-substituted alkyl, methylaminomethyl, dimethylaminomethyl, methylsulfonyloxymethyl, methoxycarbonyl, 3-cyano-4-trifluoromethylphenylcarbamoyl, carbamoyl-substituted alkyl, carboxymethyl, methoxycarbonylmethyl, methanesulfonyl, 4-cyano-3-trifluoromethylphenylcarbamoyl-substituted alkyl, carboxy-substituted alkyl, 4-methanesulfonyl-1-piperazinyl, piperazinyl, hydroxyethylcarbamoyl-substituted alkyl, or hydroxyethoxycarbonyl-substituted alkyl. R53 can be selected from the group consisting of F and H.
- 15
- 20

[0031] In an embodiment R51, comprises an alkyl chain of from 1 to 2 carbon atoms, R52 is selected from the group consisting of carbamoyl and methylcarbamoyl, and R53 is F.

[0032] A method of synthesizing a compound of formula



5 [NC53]

can include mixing 4-isothiocyanato-2-trifluoromethylbenzonitrile and *N*-methyl-4-(1-cyanocyclobutylamino)-2-fluorobenzamide in dimethylformamide to form a first mixture, heating the first mixture to form a second mixture, adding alcohol and acid to the second mixture to form a third mixture, refluxing the third mixture to form a fourth mixture, cooling the fourth mixture, combining the fourth mixture with water and extracting an organic layer, and isolating the compound from the organic layer.

[0033] A method of synthesizing the compound of claim 4 [NC54], can include mixing *N*-methyl-2-fluoro-4-(1,1-dimethyl-cyanomethyl)-aminobenzamide and 4-isothiocyanato-2-trifluoromethylbenzonitrile in DMF and heating to form a first mixture, adding an alcohol and an acid to the first mixture to form a second mixture, refluxing the second mixture, cooling the second mixture, combining the second mixture with water and extracting an organic layer, and isolating the compound from the organic layer.

[0034] A method of synthesizing the compound of claim 6 [NC55], can include mixing *N*-methyl-2-fluoro-4-(1-cyanocyclopentyl)aminobenzamide, 4-isothiocyanato-2-trifluoromethylbenzonitrile, and DMF and heating under reflux to form a first mixture, adding an alcohol and an acid to the first mixture to form a second mixture, refluxing the second mixture, cooling the second mixture, combining the second mixture with water and extracting an organic layer, and isolating the compound from the organic layer.

[0035] A method of synthesizing the compound of claim 8 [NC56], can include mixing *N,N*-

dimethyl 4-[4-(1-cyanocyclobutylamino)phenyl]butanamide, 4-isothiocyanato-2-trifluoromethyl benzonitrile, and DMF and heating under reflux to form a first mixture, adding an alcohol and an acid to the first mixture to form a second mixture, refluxing the second mixture, cooling the second mixture, combining the second mixture with water and extracting an organic layer, and isolating the compound from the organic layer.

[0036] A method of synthesizing the compound of claim 9 [NC57] can include mixing DMSO, dichloromethane, and oxalyl chloride to form a first mixture, adding 4-(4-(7-(4-cyano-3-(trifluoromethyl)phenyl)-8-oxo-6-thioxo-5,7-diazaspiro[3.4]octan-5-yl)phenyl)butanamide to the first mixture to form a second mixture, adding triethylamine to the second mixture to form a third mixture, warming the third mixture and quenching with aqueous NH₄Cl to form a fourth mixture, extracting an organic layer from the fourth mixture, isolating the compound from the organic layer.

[0037] A method can include providing at least one diarylthiohydantoin compound, measuring inhibition of androgen receptor activity for the compound and determining if the inhibition is above a first predetermined level, measuring stimulation of androgen receptor activity in hormone refractory cancer cells for the compound and determining if the stimulation is below a second predetermined level, selecting the compound if the inhibition is above the first predetermined level and the stimulation is below the second predetermined level. The predetermined levels can be those of bicalutamide. Measuring inhibition can include measuring inhibitory concentration (IC₅₀) in an AR response reporter system or a prostate specific antigen secreting system. Measuring stimulation can include measuring fold induction by increasing concentrations in an AR response reporter system or a prostate specific antigen secreting system. Measuring inhibition and/or stimulation can include measuring an effect of the compound on tumor growth in an animal. The step of measuring inhibition and/or stimulation of androgen receptor activity can include measuring the binding affinity of an androgen receptor to the compound. The step of measuring inhibition and/or stimulation of androgen receptor activity can include measuring prevention of androgen receptor recruitment to at least one of prostate specific antigen enhancer and prostate specific antigen promoter. The step of measuring inhibition and/or stimulation of androgen receptor activity can include measuring prevention of androgen receptor nuclear translocation. The step of measuring inhibition and/or stimulation of androgen receptor activity can include measuring destabilization of an androgen

receptor protein.

[0038] A method can include contacting a mammalian cell capable of expressing prostate specific antigen with a sufficient amount of a diarylthiohydantoin compound to interfere with the transcription of prostate specific antigen mRNA. The diarylthiohydantoin compound can be selected from the group consisting of NC53, NC54, NC55, NC56, and NC57. The compound can prevent formation of a transcription complex on a prostate specific antigen gene. The compound can prevent an androgen receptor protein from complexing with a prostate specific antigen gene. The compound can prevent an RNA polymerase II from complexing with a prostate specific antigen gene.

[0039] A method includes contacting a mammalian cell with a sufficient amount of a diarylthiohydantoin compound to prevent nuclear translocation of an androgen receptor protein and/or to destabilize an androgen receptor protein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0040] The following Figures present the results of pharmacological examination of certain compounds.

[0041] Figure 1 is a graph depicting that bicalutamide displays an agonistic effect on LNCaP-AR. Agonistic activities of bicalutamide in AR-overexpressed hormone refractory prostate cancer. LNCaP cells with overexpressed AR were treated with increasing concentrations of DMSO as vehicle or bicalutamide in the absence of R1881. Activities of AR response reporter were measured.

[0042] Figure 2 is a graph depicting an antagonistic assay of bicalutamide on LNCaP-AR. Agonistic activities of bicalutamide in hormone sensitive prostate cancer. LNCaP cells were treated with increasing concentrations of DMSO as vehicle or bicalutamide in the absence of R1881. Activities of AR response reporter were measured.

[0043] Figure 3 is a graph depicting the effect of compounds on LNCaP-AR.

[0044] Figure 4 is a graph depicting the inhibition effect on LNCaP-AR.

- [0045] Figure 5. Inhibitory effect on PSA expression of AR-overexpressed LNCaP xenograft model. Mice were treated with vehicle, 0.1, 1, or 10 mg per kg of example 7-3b (NC7) for 44 days orally once daily. The tumors were taken out from the mice after 44 days of treatment, tumor lysate was extracted, and PSA level in tissue lysate was determined by ELISA.
- 5 [0046] Figure 6 is a graph of tumor volume as a function of time for treatment with vehicle solution, Casodex, and NC53.
- [0047] Figure 7 is a graph of tumor size. AR overexpressing LNCaP cells were injected in the flanks of castrated SCID mice, subcutaneously. When tumors reached about 100 cubic mm, they were randomized into five groups. Each group had nine animals. After they reached this tumor volume, they were
10 given orally with either vehicle, bicalutamide or NC53 at 10 or 50 mg/kg everyday. The tumors were measured three-dimensionally, width, length and depth, using a caliper.
- [0048] Figure 8 depicts experimental results of tumor size. At day 18, the animals were imaged via an optical CCD camera, 3 hours after last dose of treatment. A ROI was drawn over the tumor for luciferase activity measurement in photon/second. The right panels is a representation of the ROIs measurements.
- 15 [0049] Figure 9 is a graph depicting the pharmacokinetic curves of NC53 from intravenous (upper curve) and oral administration (lower curve).
- [0050] Figure 10 is a graph of fluorescence absorbance as a function of the logarithm of concentration, which reflects the binding affinities of several compounds to rat androgen receptor.
- [0051] Figure 11 presents images reflecting the state of complexation of androgen receptor and
20 RNA polymerase II to PSA enhancer and to PSA promoter when Casodex or NC53 are added.
- [0052] Figure 12 presents images reflecting that androgen receptor translocates into the nucleus in the presence of Casodex, but not in the presence of NC53.
- [0053] Figure 13 presents images reflecting that androgen receptor translocates into the nucleus in the presence of Casodex, but not in the presence of NC53.
- 25 [0054] Figure 14 presents images reflecting that androgen receptor protein is degraded in the presence of NC53.

[0055] Figure 15 is a chart depicting prostate weight after treatment with various compounds. 10, 25, or 50 mg of compound per kilogram body weight were administered per day, as indicated by the label of a bar. The compounds were administered to healthy FVB mice. After treatment with compound for 14 days, the urogenital tract weight was determined by removing and weighing the semi-vesicles, prostate, and bladder. Three mice were administered a given compound to obtain the data presented by a bar in the chart. A set of mice was not treated with a compound: data are presented in the bar labeled "untreated". Another set of mice was treated only with vehicle solution: data are presented in the bar labeled "vehicle".

[0056] Figure 16 is a graph presenting a PSA assay performed along with the experimental protocol presented in Fig. 6.

10 [0057] Figure 17 is a graph presenting the effect of various dose regimens of NC53 on tumor volume.

[0058] Figure 18 is a graph presenting the rate of photon emission associated with luciferase activity at day 17 relative to the rate at day 0 after treatment with NC53 at doses of 0.1, 1, and 10 mg per kilogram body weight per day and without treatment with NC53.

15 [0059] Figure 19 presents the results of an experiment in which SCID mice were injected with the LN-AR (HR) cell line to induce tumor growth. One set of mice were treated with the compound NC53 at a dose of 10 mg per kilogram body weight per day; the other set of mice were treated only with vehicle solution. (A) The relative tumor volume as a function of time shown for each set of mice. (B) Images of each set of mice with photon emission associated with luciferase activity at day 31 shown as color contours.
20 (C) Rate of photon emission associated with luciferase activity shown at several times for each set of mice.

[0060] Figure 20 is a graph presenting PSA absorbance associated with LN-AR cells treated with various concentrations of NC53, NC54, NC55, and NC57 and vehicle solution.

[0061] Figure 21 is a graph presenting PSA absorbance associated with LN-CaP cells treated with various concentrations of NC7, NC48, NC53, bicalutamide, and DMSO.

25 [0062] Figure 22 presents results of an experiment conducted with wild type nontransgenic mice (WT), castrated luciferase transgenic mice (Cast), and non-castrated luciferase transgenic mice (Intact). Data are shown for castrated luciferase transgenic mice treated with an implanted testosterone pellet yielding 12.5 mg per kilogram body weight with a 90 day release period (T/Cast), and data are shown for non-castrated

luciferase transgenic mice treated with an implanted testosterone pellet yielding 12.5 mg per kilogram body weight with a 90 day release period (Intact+T). Data are shown for castrated luciferase transgenic mice treated with the implanted testosterone pellet and with bicalutamide (BIC+T/Cast) or with NC53 (NC53+T/Cast) at 10 mg per kilogram body weight per day. (A) Urogenital tract weight at 14 days. (B) Photon emission rate at 14 days. In all cases, a hormone refractory disease state was not induced.

[0063] Figure 23 is a graph depicting PSA absorbance measured for LN-AR cells after treatment with various doses of several compounds.

[0064] Figure 24 presents a table providing several characteristics of compounds. Figure 15 also presents a graph providing the pharmacokinetic characteristics of several compounds in terms of compound serum concentration as a function of time.

[0065] Figure 25 is a graph of luciferase activity of the LIAR cell line dosed with various compounds administered at concentrations ranging from 125 nmol to 1000 nmol.

DETAILED DESCRIPTION

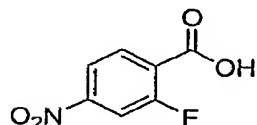
[0066] Embodiments of the invention are discussed in detail below. In describing embodiments, specific terminology is employed for the sake of clarity. However, the invention is not intended to be limited to the specific terminology so selected. A person skilled in the relevant art will recognize that other equivalent parts can be employed and other methods developed without parting from the spirit and scope of the invention. All references cited herein are incorporated by reference as if each had been individually incorporated.

Synthesis of Diarylhydantoin Compounds

Example 56 [NC54]

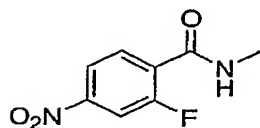
[0067] In the following, air or moisture sensitive reactions were conducted under argon atmosphere using oven-dried glassware and standard syringe/septa techniques. The reactions were monitored with a SiO₂ TLC plate under UV light (254 nm) followed by visualization with a *p*-anisaldehyde or ninhydrin staining

solution. Column chromatography was performed on silica gel 60. ^1H NMR spectra were measured at 400 MHz in CDCl_3 unless stated otherwise and data were reported as follows in ppm (δ) from the internal standard (TMS, 0.0 ppm): chemical shift (multiplicity, integration, coupling constant in Hz.).



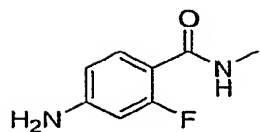
5 Formula 37

[0068] Periodic acid (1.69 g, 7.41 mmol) was dissolved in acetonitrile (25 mL) by vigorous stirring, and then chromium trioxide (0.16 g, 1.60 mmol) was dissolved into the solution. 2-Fluoro-4-nitrotoluene (0.33 g, 2.13 mmol) was added to the above solution with stirring. A white precipitate formed immediately with exothermic reaction. After 1 h of stirring, the supernatant liquid of the reaction mixture was decanted to
10 a flask, and the solvent was removed by evaporation. The residues were extracted with methylene chloride (2×30 mL) and water (2×30 mL). The organic layer was dried over MgSO_4 , and concentrated to give 2-Fluoro-4-nitrobenzoic acid (Formula 37) (0.32 mg, 81%) as a white solid. ^1H NMR δ 8.06 (ddd, 1 H, $J=9.9$, 2.2 and 0.3), 8.13 (ddd, 1 H, $J=8.6$, 2.2 and 0.9), 8.25 (ddd, 1 H, $J=8.6$, 7.0 and 0.3).



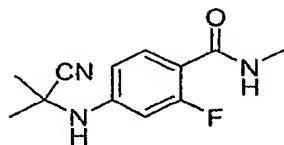
15 Formula 38

[0069] Thionyl chloride (0.15 g, 1.30 mmol) was added slowly to a solution of 2-fluoro-4-nitrobenzoic acid (Formula 37) (0.20 g, 1.10 mmol) in DMF (5 mL) cooled at -5°C . The mixture was stirred for an additional 1 hour at -5°C . Excess methylamine (freshly distilled from its 40% aqueous solution) was added to the reaction medium. The second mixture was stirred for an additional 1 hour. Ethyl acetate (50
20 mL) was added to the mixture, which was washed with brine (2 × 50 mL). The organic layer was dried over MgSO_4 , and concentrated to yield *N*-Methyl-2-fluoro-4-nitrobenzamide (Formula 38) (0.18 g, 85%) as a yellowish solid. ^1H NMR (acetone- d_6) δ 3.05 (d, 3 H, $J=4.3$), 6.31 (dd, 1 H, $J=13.5$ and 2.1), 6.40 (dd, 1H, $J=8.6$ and 2.1), 7.64 (dd, 1H, $J=8.6$ and 8.6).



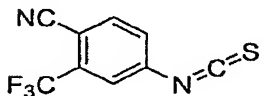
Formula 39

[0070] A mixture of *N*-Methyl-2-fluoro-4-nitrobenzamide (Formula 38) (0.18 g, 0.91 mmol) and iron (0.31 g, 5.60 mmol) in ethyl acetate (5 mL) and acetic acid (5 mL) was refluxed for 1 h. The solid particles were filtered off. The filtrate was washed with water and extracted with ethyl acetate. The organic layer was dried over MgSO₄, concentrated and the residue was purified with SiO₂ column chromatography (dichloromethane:acetone, 95:5) to give *N*-Methyl-2-fluoro-4-aminobenzamide (Formula 39) (0.14 g, 92%) as an off-white solid. ¹H NMR (acetone-*d*₆) δ 2.86 (d, 3 H, *J*=4.3), 5.50 (br s, 2 H), 6.37 (dd, 1 H, *J*=14.7 and 2.1), 6.50 (dd, 1H, *J*=8.6 and 2.1), 7.06 (br s, 1H), 7.68 (dd, 1H, *J*=8.8 and 8.8).



Formula 40

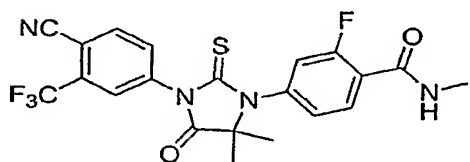
[0071] A mixture of *N*-Methyl-2-fluoro-4-aminobenzamide (Formula 39) (96 mg, 0.57 mmol), acetone cyanohydrin (0.3 mL, 3.14 mmol) and magnesium sulfate (50 mg) was heated to 80 °C and stirred for 12 h. To the medium was added ethyl acetate (25 mL) and then washed with water (2 × 25 mL). The organic layer was dried over MgSO₄ and concentrated and the residue was purified with SiO₂ column chromatography (dichloromethane:acetone, 95:5) to give *N*-Methyl-2-fluoro-4-(1,1-dimethyl-2-cyanomethyl)aminobenzamide (Formula 40) (101 mg, 75%) as a white solid. ¹H NMR δ 1.74 (s, 6 H), 2.98 (dd, 3 H, *J*=4.8 and 1.1), 6.58 (dd, 1 H, *J*=14.6 and 2.3), 6.63 (dd, 1 H, *J*=8.7 and 2.3), 6.66 (br s, 1 H), 7.94 (dd, 1 H, *J*=8.7 and 8.7).



Formula 41

[0072] 4-Amino-2-trifluoromethylbenzonitrile (2.23 g, 12 mmol) was added portionwise over 15

min into a well-stirred heterogeneous mixture of thiophosgene (1 mL, 13 mmol) in water (22 mL) at room temperature. Stirring was continued for an additional 1 h. The reaction medium was extracted with chloroform (3 × 15 ml). The combined organic phase was dried over MgSO₄ and evaporated to dryness under reduced pressure to yield desired product 4-Isothiocyanato-2-trifluoromethylbenzonitrile (Formula 41) as brownish solid and was used as such for the next step (2.72 g, 11.9 mmol, 99%). ¹H NMR δ 7.49 (dd, 1 H, *J*=8.3 and 2.1), 7.59 (d, 1 H, *J*=2.1), 7.84 (d, 1 H, *J*=8.3).

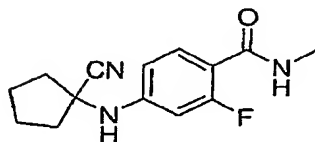


NC54 (Formula 42)

56-1) NC54

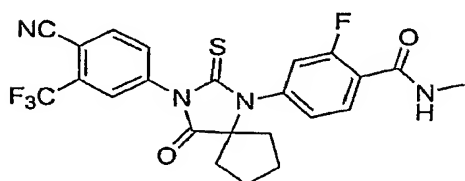
[0073] A mixture of *N*-Methyl-2-fluoro-4-(1,1-dimethyl-cyanomethyl)-aminobenzamide (Formula 40) (30 mg, 0.13 mmol) and 4-Isothiocyanato-2-trifluoromethylbenzonitrile (Formula 41) (58 mg, 0.26 mmol) in DMF (1 mL) was heated under microwave irradiation at 100 °C for 11 hours. To this mixture was added methanol (20 mL) and *aq.* 1 N HCl (5 mL). The second mixture was refluxed for 1.5 h. After being cooled to room temperature, the reaction mixture was poured into cold water (50 mL) and extracted with ethyl acetate (50 mL). The organic layer was dried over MgSO₄, concentrated and the residue was purified with SiO₂ column chromatography (dichloromethane:acetone, 95:5) to give NC54 (Formula 42) (15 mg, 25%) as a colorless crystal. ¹H NMR δ 1.61 (s, 6 H), 3.07 (d, 3 H, *J*=4.1), 6.71 (m, 1 H), 7.15 (dd, 1H, *J*=11.7 and 2.0), 7.24 (dd, 1H, *J*=8.4 and 2.0), 7.83 (dd, 1H, *J*=8.2 and 2.1), 7.95 (d, 1H, *J*=2.1), 7.99 (d, 1H, *J*=8.2), 8.28 (dd, 1H, *J*=8.4 and 8.4).

Example 57



Formula 43

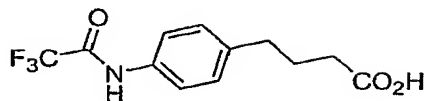
[0074] A mixture of *N*-Methyl-2-fluoro-4-aminobenzamide (Formula 39) (62 mg, 0.37 mmol), cyclopentanone (0.07 mL, 0.74 mmol) and TMSCN (0.1 mL, 0.74 mmol) was heated to 80 °C and stirred for 13 h. To the medium was added ethyl acetate (2 × 20 mL) and then washed with water (2 × 20 mL). The organic layer was dried over MgSO₄ and concentrated and the residue was purified with silica gel column chromatography (dichloromethane:acetone, 95:5) to give *N*-Methyl 2-fluoro-4-(1-cyanocyclopentyl)aminobenzamide (Formula 43) (61 mg, 63%) as a white solid. ¹H NMR δ 7.95 (dd, 1H, *J* = 8.8, 8.8 Hz), 6.65 (br s, 1H), 6.59 (dd, 1H, *J* = 8.8, 2.3 Hz), 6.50 (dd, 1H, *J* = 14.6, 2.3 Hz), 4.60 (br s, 1H), 2.99 (dd, 3H, *J* = 4.8, 1.1 Hz), 2.36-2.45 (m, 2H), 2.10-2.18 (m, 2H), 1.82-1.95 (m, 4H).



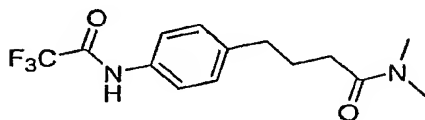
NC55 (Formula 44)

57-1) NC55

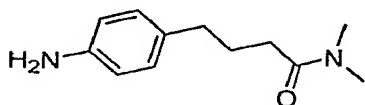
[0075] A mixture of *N*-Methyl 2-fluoro-4-(1-cyanocyclopentyl)aminobenzamide (Formula 43) (57 mg, 0.22 mmol) and 4-isothiocyanato-2-trifluoromethyl benzonitrile (0.15 g, 0.65 mmol) in DMF (3 mL) was heated under microwave irradiation (open vessel) at 130 °C for 12 hours. To this mixture was added methanol (20 mL) and *aq.* 1 N HCl (5 mL). The second mixture was refluxed for 1.5 h. After being cooled to room temperature, the reaction mixture was poured into cold water (50 mL) and extracted with ethyl acetate (50 mL). The organic layer was dried over MgSO₄, concentrated and the residue was purified with silica gel column chromatography (dichloromethane:acetone, 95:5) to give 4-(3-(4-Cyano-3-(trifluoromethyl)phenyl)-4-oxo-2-thioxo-1,3-diazaspiro[4.4]nonan-1-yl)-2-fluoro-*N*-methylbenzamide, NC55 (Formula 44) (8 mg, 7%) as a pale yellowish solid. ¹H NMR δ 8.28 (dd, 1H, *J* = 8.4, 8.4 Hz), 7.98 (d, 1H, *J* = 8.3 Hz), 7.96 (d, 1H, *J* = 1.8 Hz), 7.84 (dd, 1H, *J* = 8.3, 1.8 Hz), 7.27 (dd, 1H, *J* = 8.4, 1.8 Hz), 7.17 (dd, 1H, *J* = 11.7, 1.8 Hz), 6.67-6.77 (m, 1H), 3.07 (d, 3H, *J* = 4.3 Hz), 2.32-2.41 (m, 2H), 2.13-2.21 (m, 2H), 1.85-1.96 (m, 2H), 1.49-1.59 (m, 2H).

Example 58**Formula 45**

[0076] Trifluoroacetic anhydride (0.85 mL, 6.14 mmol) was added to a solution of 4-(4-aminophenyl)butyric acid (0.5 g, 2.79 mmol) in chloroform (10 mL) at 0 °C. The mixture was warmed to room temperature and stirred for 3 hours. The mixture was partitioned with chloroform (20 mL) and water (20 mL). The organic layer was dried over MgSO₄, concentrated and the residue was purified with silica gel column chromatography (dichloromethane:acetone, 9:1) to give 4-[4-(2,2,2-Trifluoroacetylaminophenyl)]butanoic acid (Formula 45) (0.53 g, 69%). ¹H NMR δ 7.81 (br s, 1H), 7.48 (d, 2H, *J* = 8.5 Hz), 7.22 (d, 2H, *J* = 8.5 Hz), 2.68 (t, 2H, *J* = 7.5 Hz), 2.38 (t, 2H, *J* = 7.5 Hz), 1.96 (p, 2H, *J* = 7.5 Hz).

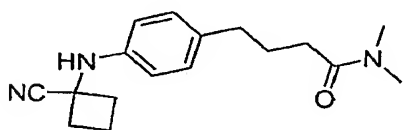
**Formula 46**

[0077] Thionyl chloride (71 mg, 0.60 mmol) was added slowly to a solution of 4-[4-(2,2,2-Trifluoroacetylaminophenyl)]butanoic acid (Formula 45) (0.15 g, 0.55 mmol) in DMF (5 mL) cooled at -5 °C. The mixture was stirred for an additional 1 hour at -5 °C. Excess dimethylamine (freshly distilled from its 40% aqueous solution) was added to the reaction medium. The second mixture was stirred for an additional 1 hour. Ethyl acetate (50 mL) was added to the mixture, which was washed with brine (2 × 50 mL). The organic layer was dried over MgSO₄, and concentrated to yield *N,N*-Dimethyl 4-[4-(2,2,2-Trifluoroacetylaminophenyl)]butanamide (Formula 46) (0.17 g, quant.) as a yellowish solid. ¹H NMR δ 9.70 (br s, 1H), 7.55 (d, 2H, *J* = 8.6 Hz), 7.11 (d, 2H, *J* = 8.6 Hz), 2.91 (s, 3H), 2.89 (s, 3H), 2.60 (t, 2H, *J* = 7.7 Hz), 2.27 (t, 2H, *J* = 7.7 Hz), 1.89 (p, 2H, *J* = 7.7 Hz).



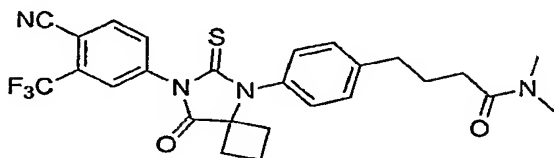
Formula 47

[0078] 1 N NaOH solution (3 mL) was added to a solution of *N,N*-Dimethyl 4-[4-(2,2,2-Trifluoroacetyl-amino)phenyl]butanamide (Formula 46) (0.17 g, 0.55 mmol) in methanol (2 mL) at room temperature. The mixture was stirred for 14 hour. The mixture was partitioned with chloroform (25 mL) and water (25 mL). The organic layer was dried over MgSO_4 , and concentrated and the residue was purified with silica gel column chromatography (dichloromethane:acetone, 9:1) to give *N,N*-Dimethyl 4-(4-aminophenyl)butanamide (Formula 47) (74 mg, 66%) as a white solid. ^1H NMR δ 6.97 (d, 2H, $J = 8.3$ Hz), 6.61 (d, 2H, $J = 8.3$ Hz), 3.56 (br s, 2H), 2.92 (s, 6 H), 2.56 (t, 2H, $J = 7.7$ Hz), 2.28 (t, 2H, $J = 7.7$ Hz), 1.91 (p, 2H, $J = 7.7$ Hz).



Formula 48

[0079] A mixture of *N,N*-Dimethyl 4-(4-aminophenyl)butanamide (Formula 47) (74 mg, 0.36 mmol), cyclobutanone (54 mg, 0.78 mmol) and TMSCN (77 mg, 0.78 mmol) was heated to 80°C and stirred for 15 h. To the medium was added ethyl acetate (2×20 mL) and then washed with water (2×20 mL). The organic layer was dried over MgSO_4 and concentrated and the residue was purified with silica gel column chromatography (dichloromethane:acetone, 9:1) to give *N,N*-Dimethyl 4-[4-(1-cyanocyclobutylamino)phenyl]butanamide (Formula 48) (58 mg, 57%) as a white solid. ^1H NMR δ 7.07 (d, 2H, $J = 8.5$ Hz), 6.59 (d, 2H, $J = 8.5$ Hz), 3.94 (br s, 1H), 2.94 (s, 3H), 2.93 (s, 3H), 2.75-2.83 (m, 2H), 2.60 (t, 2H, $J = 7.6$ Hz), 2.33-2.42 (m, 2H), 2.30 (t, 2H, $J = 7.6$ Hz), 2.11-2.28 (m, 2H), 1.93 (p, 2H, $J = 7.6$ Hz).

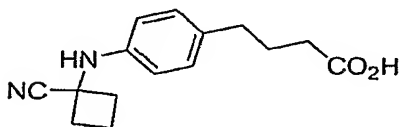


NC56 Formula 49

[0080] A mixture of *N,N*-Dimethyl 4-[4-(1-cyanocyclobutylamino)phenyl]butanamide (Formula 48) (58 mg, 0.20 mmol) and 4-isothiocyanato-2-trifluoromethyl benzonitrile (74 mg, 0.32 mmol) in DMF (3 mL)

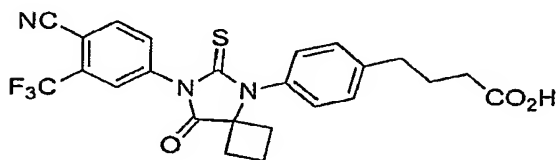
was heated under reflux for 2 hours. To this mixture was added methanol (20 mL) and *aq.* 1 N HCl (5 mL). The second mixture was refluxed for 1.5 h. After being cooled to room temperature, the reaction mixture was poured into cold water (50 mL) and extracted with ethyl acetate (50 mL). The organic layer was dried over MgSO_4 , concentrated and the residue was purified with silica gel column chromatography (dichloromethane:acetone, 95:5) to give 4-(4-(7-(4-Cyano-3-(trifluoromethyl)phenyl)-8-oxo-6-thioxo-5,7-diazaspiro[3.4]octan-5-yl)phenyl)-*N,N*-dimethylbutanamide, NC56 (Formula 49) (44 mg, 42%) as a pale yellowish solid. ^1H NMR δ 7.98 (s, 1H), 7.97 (d, 1H, $J = 8.2$ Hz), 7.86 (d, 1H, $J = 8.2$ Hz), 7.42 (d, 2H, $J = 8.3$ Hz), 7.22 (d, 2H, $J = 8.3$ Hz), 2.99 (s, 3H), 2.96 (s, 3H), 2.78 (t, 2H, $J = 7.5$ Hz), 2.62-2.70 (m, 2H), 2.52-2.63 (m, 2H), 2.40 (t, 2H, $J = 7.5$ Hz), 2.15-2.30 (m, 1H), 2.04 (p, 2H, $J = 7.5$ Hz), 1.62-1.73 (m, 1H).

10 Example 59



Formula 50

[0081] A mixture of 4-(4-aminophenyl)butyric acid (0.20 g, 1.12 mmol), cyclobutanone (0.17 mL, 2.23 mmol) and TMSCN (0.30 mL, 2.23 mmol) was heated to 80 °C and stirred for 13 h. To the medium was added ethyl acetate (2 × 30 mL) and then washed with water (2 × 30 mL). The organic layer was dried over MgSO_4 and concentrated and the residue was purified with silica gel column chromatography (dichloromethane:acetone, 9:1) to give 4-[4-(1-Cyanocyclobutylamino)phenyl]butanoic acid (Formula 50) (0.21 g, 74%) as a yellowish solid. ^1H NMR δ 7.06 (d, 2H, $J = 8.6$ Hz), 6.59 (d, 2H, $J = 8.6$ Hz), 2.75-2.83 (m, 2H), 2.59 (t, 2H, $J = 7.5$ Hz), 2.37 (t, 2H, $J = 7.5$ Hz), 2.33-2.42 (m, 2H), 2.11-2.28 (m, 2H), 1.92 (p, 2H, $J = 7.5$ Hz).

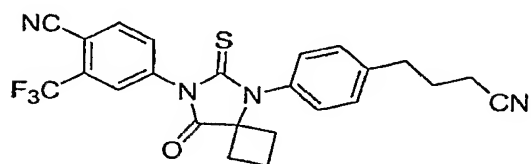


Formula 51

[0082] A mixture of 4-[4-(1-Cyanocyclobutylamino)phenyl]butanoic acid (Formula 50) (0.21 g,

0.83 mmol) and 4-isothiocyanato-2-trifluoro benzonitrile (0.25 g, 1.08 mmol) in toluene (10 mL) was heated under reflux for 1 hours. To this mixture was added *aq.* 1 N HCl (5 mL). The second mixture was refluxed for 1.5 h. After being cooled to room temperature, the reaction mixture was poured into cold water (50 mL) and extracted with ethyl acetate (50 mL). The organic layer was dried over MgSO₄, concentrated and the residue was purified with silica gel column chromatography (dichloromethane:acetone, 95:5) to give 4-(4-(7-(4-Cyano-3-(trifluoromethyl)phenyl)-8-oxo-6-thioxo-5,7-diazaspiro[3.4]octan-5-yl)phenyl)butanoic acid, NC122 (Formula 51) (60 mg, 15%). ¹H NMR δ 7.98 (d, 1H, *J* = 1.8 Hz), 7.97 (d, 1H, *J* = 8.3 Hz), 7.86 (dd, 1H, *J* = 8.3, 1.8 Hz), 7.42 (d, 2H, *J* = 8.5 Hz), 7.24 (d, 2H, *J* = 8.5 Hz), 2.79 (t, 2H, *J* = 7.5 Hz), 2.62-2.68 (m, 2H), 2.51-2.59 (m, 2H), 2.47 (t, 2H, *J* = 7.5 Hz), 2.14-2.26 (m, 1H), 2.06 (p, 2H, *J* = 7.5 Hz), 1.60-1.70 (m, 1H).

Example 61



NC57 Formula 53

A solution of DMSO (0.01 mL, 0.12 mmol) in dry dichloromethane (1 mL) was added to a stirred solution of oxalyl chloride (0.01 mL, 0.09 mmol) in dry dichloromethane (2 mL) at -78 °C. After 15 min, a dichloromethane solution of 4-(4-(7-(4-Cyano-3-(trifluoromethyl)phenyl)-8-oxo-6-thioxo-5,7-diazaspiro[3.4]octan-5-yl)phenyl)butanamide, NC47 (Formula 52) (35 mg, 0.07 mmol) was added to the reaction mixture. Stirring was continued for 20 min at -78 °C, and then triethylamine (0.03 mL, 0.22 mmol) was added. After 30 min at -78 °C, the reaction mixture was warmed to room temperature and then reaction was quenched with saturated *aq.* NH₄Cl solution. The reaction mixture was diluted with dichloromethane, and extracted with dichloromethane. The organic layer was dried over MgSO₄, concentrated and chromatographed (dichloromethane:acetone, 95:5) to yield 4-(5-(4-(3-Cyanopropyl)phenyl)-8-oxo-6-thioxo-5,7-diazaspiro[3.4]octan-7-yl)-2-(trifluoromethyl)benzonitrile, NC57 (Formula 53) (29 mg, 87%) as a viscous oil. ¹H NMR δ 7.98 (d, 1H, *J* = 1.8 Hz), 7.98 (d, 1H, *J* = 8.3 Hz), 7.86 (dd, 1H, *J* = 8.3, 1.8 Hz), 7.43 (d, 2H, *J* = 8.4 Hz), 7.27 (d, 2H, *J* = 8.4 Hz), 2.90 (t, 2H, *J* = 7.3 Hz), 2.63-2.73 (m, 2H), 2.52-2.62 (m, 2H), 2.42 (t, 2H, *J* = 7.3 Hz), 2.18-2.30 (m, 1H), 2.07 (p, 2H, *J* = 7.3 Hz), 1.63-1.73 (m, 1H).

[0083]

One skilled in the art could modify and/or combine the syntheses described herein to make

other diarylhydantoin compounds.

Pharmacological examination of the compounds

[0084] Compounds for which synthetic routes are described above were identified through screening on hormone refractory prostate cancer cells for antagonistic and agonistic activities against AR
5 utilizing screening procedures similar to those in PCT applications US04/42221 and US05/05529, which are hereby incorporated by reference. A number of compounds exhibited potent antagonistic activities with minimal agonistic activities for over expressed AR in hormone refractory prostate cancer.

In vitro biological assay

Effect of compounds on AR by a reporter assay

10 [0085] The compounds were subjected to tests using an artificial AR response reporter system in a hormone refractory prostate cancer cell line. In this system, the prostate cancer LNCaP cells were engineered to stably express about 5-fold higher level of AR than endogenous level. The exogenous AR has similar properties to endogenous AR in that both are stabilized by a synthetic androgen R1881. The AR-over
15 expressed cells were also engineered to stably incorporate an AR response reporter and the reporter activity of these cells shows features of hormone refractory prostate cancer. It responds to low concentration of a synthetic androgen R1881, is inhibited only by high concentrations of bicalutamide (see Table 1), and displays agonistic activity with bicalutamide (Figure 1 and Table 2). Consistent with published data, bicalutamide inhibited AR response reporter and did not have agonistic activity in hormone sensitive prostate cancer cells (Figure 2).

20 Table 1

[0086] We examined the antagonistic activity of the compounds for which the synthesis is described above in the presence of 100 pM of R1881. Engineered LNCaP cells (LNCaP-AR, also abbreviated LN-AR) were maintained in Iscove's medium containing 10% fetal bovine serum (FBS). Two days prior to drug
25 treatment, the cells were grown in Iscove's medium containing 10% charcoal-stripped FBS (CS-FBS) to deprive of androgens. The cells were split and grown in Iscove's medium containing 10% CS-FBS with 100 pM of R1881 and increasing concentrations of test compounds. After two days of incubation, reporter activities were assayed.

[0087] Table 1 lists the IC₅₀ of these compounds to inhibit AR in hormone refractory prostate cancer. The control substance bicalutamide has an IC₅₀ of 889 nM. Most of the compounds identified (diarylthiohydantoin)s have IC₅₀s between 100 to 200 nM in inhibiting AR in hormone refractory prostate cancer. In contrast, antiandrogenic compounds listed as examples in US patent no. 5,705,654, such as
 5 examples 30-2, 30-3, 31-2, 31-3, and 24-3 (NC24-NC28) have no inhibitory activities on AR in this system.

Antagonistic activities against AR in hormone refractory prostate cancer, measured by an AR response reporter and by endogenous PSA expression.

| | | IC ₅₀ (nM) | IC ₅₀ (nM) |
|-----------------------|--|-----------------------|-----------------------|
| Example | Name | Reporter | PSA |
| Bicalutamide | N-[4-cyano-3-(trifluoromethyl)phenyl]-3-[(4-fluorophenyl)sulfonyl]-2-hydroxy-2-methylpropanamide | 889 | >1000 |
| Comparative | | | |
| 29 | 4-[3-(4-hydroxybutyl)-4,4-dimethyl-5-oxo-2-thioxoimidazolidin-1-yl]-2-trifluoromethylbenzonitrile | No(*) | No |
| Comparative | | | |
| 6-2 (6b) [NC10] | 4-[3-phenyl-4,4-dimethyl-5-oxo-2-thioxoimidazolidin-1-yl]-2-trifluoromethylbenzonitrile | 149 | n/a (**) |
| 5-3b (5c) [NC2] | 4-[3-(4-methylphenyl)-4,4-dimethyl-5-oxo-2-thioxoimidazolidin-1-yl]-2-trifluoromethylbenzonitrile | 125 | 132 |
| 3-3 (3c) [NC3] | 4-[3-(4-hydroxyphenyl)-4,4-dimethyl-5-oxo-2-thioxoimidazolidin-1-yl]-2-trifluoromethylbenzonitrile | 137 | 122 |
| 2-4 (2d) [NC4] | 4-[3-(4-aminophenyl)-4,4-dimethyl-5-oxo-2-thioxoimidazolidin-1-yl]-2-trifluoromethylbenzonitrile | 273 | n/a |
| 4 (4a) [NC5] | Chloroacetic acid 4-[3-(4-cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]phenyl ester | 131 | n/a |
| 8-2 (8b) [NC6] | 4-(4-Oxo-2-thioxo-1-(4-methylphenyl)-1,3-diazaspiro[4.4]non-3-yl)-2-trifluoromethylbenzonitrile | 147 | n/a |
| 7-3b (7c) [NC7] | 4-(8-oxo-6-thioxo-5-(4-methylphenyl)-5,7-diazaspiro[3.4]oct-7-yl)-2-trifluoromethylbenzonitrile | 124 | 128 |

| | | | |
|-----------------------------------|---|-----|------|
| 9-3 (9c) [NC8] | 4-(4-Oxo-2-thioxo-1-(4-methylphenyl)-1,3-diazaspiro[4.5]dec-3-yl)-2-trifluoromethylbenzonitrile | 194 | n/a |
| 10-3 (10c) [NC9] | 4-(4-oxo-2-thioxo-1-(4-methylphenyl)-1,3-diazaspiro[4.5]undec-3-yl)-2-trifluoromethylbenzonitrile | 232 | n/a |
| 28 Comparative (28a) [NC10] | 4-(8-methyl-4-oxo-2-thioxo-1,3,8-triazaspiro[4.5]dec-3-yl)-2-trifluoromethylbenzonitrile | No | n/a |
| 27-3 (27c) [NC11] | 4-(8-methyl-4-oxo-2-thioxo-1-(4-methylphenyl)-1,3,8-triazaspiro[4.5]dec-3-yl)-2-trifluoromethylbenzonitrile | 638 | n/a |
| 26 (26a) [NC12] | 4-[1-(4-cyanophenyl)-4-oxo-2-thioxo-1,3-diazaspiro[4.4]non-3-yl]-2-trifluoromethylbenzonitrile | 469 | n/a |
| 25 (25a) [NC13] | 4-[1-(4-nitrophenyl)-4-oxo-2-thioxo-1,3-diazaspiro[4.4]non-3-yl]-2-trifluoromethylbenzonitrile | 498 | n/a |
| 12-2 (12b) [NC15] | 4-(8-oxo-6-thioxo-5-(4-biphenyl)-5,7-diazaspiro[3.4]oct-7-yl)-2-trifluoromethylbenzonitrile | 283 | n/a |
| 11-2 (11b) [NC16] | 4-(8-oxo-6-thioxo-5-(4-hydroxyphenyl)-5,7-diazaspiro[3.4]oct-7-yl)-2-trifluoromethylbenzonitrile | 162 | n/a |
| 17 (17a) [NC17] | 4-[3-(4-hydroxyphenyl)-4,4-dimethyl-2,5-dithioxoimidazolidin-1-yl]-2-trifluoromethylbenzonitrile | 278 | 287 |
| 18 (18a) [NC18] | 4-[3-(4-hydroxyphenyl)-4,4-dimethyl-2,5-dioxoimidazolidin-1-yl]-2-trifluoromethylbenzonitrile | 369 | 511 |
| 22-2 (22b) [NC19] | 2-[3-(4-cyano-3-trifluoromethylphenyl)-5,5-dimethyl-4-oxo-2-thioxoimidazolidin-1-yl]benzoic acid | 523 | >500 |
| 20-2 (20b) [NC20] | 4-(4,4-dimethyl-5-oxo-2-thioxo-3-(4-trifluoromethylphenyl)imidazolidin-1-yl)-2-trifluoromethylbenzonitrile | 143 | 144 |
| 21-2 (21b) [NC21] | 4-(4,4-bis(chloromethyl)-5-oxo-2-thioxo-3-(4-methylphenyl)imidazolidin-1-yl)-2-trifluoromethylbenzonitrile | 521 | >500 |
| 19-2 | 4-(4-fluoromethyl-4-methyl-5-oxo-2-thioxo-3-(4- | 126 | 129 |

| | | | |
|-------------------------------------|---|-------|-----|
| (19b) [NC22] | methylphenyl)imidazolidin-1-yl)-2-trifluoromethylbenzonitrile | | |
| 23-2 (23b) [NC23] | 4-(8-oxo-6-thioxo-5-(2-methylphenyl)-5,7-diazaspiro[3.4]oct-7-yl)-2-trifluoromethylbenzonitrile | 258 | 232 |
| 30-2 Comparative (30b) [NC24] | 4-(5-methyl-8-oxo-6-thioxo-5,7-diazaspiro[3.4]oct-7-yl)-2-trifluoromethylbenzonitrile | No | No |
| 30-3 Comparative (30c) [NC25] | 4-(5-methyl-6,8-dioxo-5,7-diazaspiro[3.4]oct-7-yl)-2-trifluoromethylbenzonitrile | No | No |
| 31-2 Comparative (31b) [NC26] | 4-(1-methyl-4-oxo-2-thioxo-1,3-diazaspiro[4.4]non-3-yl)-2-trifluoromethylbenzonitrile | No | No |
| 31-3 Comparative (31c) [NC27] | 4-(1-methyl-2,4-dioxo-1,3-diazaspiro[4.4]non-3-yl)-2-trifluoromethylbenzonitrile | No | No |
| 24-3 Comparative (24c) [NC28] | 4-(4-oxo-2-thioxo-1,3-diazaspiro[4.4]non-3-yl)-2-trifluoromethylbenzonitrile | No | No |
| 15-2 (15b) [NC29] | 4-[4,4-dimethyl-3-(4-pyridin-2-yl)-5-oxo-2-thioxoimidazolidin-1-yl]-2-trifluoromethylbenzonitrile | 723 | n/a |
| 14-2 (14b) [NC30] | 4-[4,4-dimethyl-3-(4-methylpyridin-2-yl)-5-oxo-2-thioxoimidazolidin-1-yl]-2-trifluoromethylbenzonitrile | 457 | n/a |
| 16-2 Comparative (16b) [NC31] | 4-[5-(5-methyl-2H-pyrazol-3-yl)-8-oxo-6-thioxo-5,7-diazaspiro[3.4]oct-7-yl]-2-trifluoromethylbenzonitrile | >1000 | n/a |
| 13-2 (12b) [NC32] | 4-(8-oxo-6-thioxo-5-(4-biphenyl)-5,7-diazaspiro[3.4]oct-7-yl)-2-trifluoromethylbenzonitrile | >1000 | n/a |
| 32 (32a) [NC33] | 4-(8-methylimino-6-thioxo-5-p-tolyl-5,7-diazaspiro[3.4]oct-7-yl)-2-trifluoromethylbenzonitrile | 222 | 421 |
| 33 (33a) [NC34] | 1-[3-(4-cyano-3-trifluoromethylphenyl)-5,5-dimethyl-2-thioxo-1-p-tolyl-imidazolidin-4-ylidene]-3-ethyl- | 157 | 239 |

| | | | |
|-------------------------|---|-----|-----|
| | thiourea | | |
| 34 (34a) [NC35] | 1-[7-(4-cyano-3-trifluoromethyl-phenyl)-6-thioxo-5-p-tolyl-5,7-diaza-spiro[3.4]oct-8-ylidene]-3-phenyl-thiourea | 176 | 276 |
| 35 (35a) [NC36] | 1-(4-Cyano-3-trifluoromethyl-phenyl)-3-[7-(4-cyano-3-trifluoromethyl-phenyl)-6-thioxo-5-p-tolyl-5,7-diaza-spiro[3.4]oct-8-ylidene]-thiourea | 144 | 158 |
| 36-2 (36b) [NC37] | 4-[8-(4-hydroxymethyl-phenyl)-5-oxo-7-thioxo-6-aza-spiro[3.4]oct-6-yl]-2-trifluoromethyl-benzonitrile | 311 | 337 |
| 37 (37a) [NC38] | 4-[5-(4-formylphenyl)-8-oxo-6-thioxo-5,7-diazaspiro[3.4]oct-7-yl]-2-trifluoromethyl-benzonitrile | n/a | 263 |
| 38 (38a) [NC39] | 4-{5-[4-(1-hydroxyethyl)-phenyl]-8-oxo-6-thioxo-5,7-diazaspiro[3.4]oct-7-yl}-2-trifluoromethyl-benzonitrile | n/a | 187 |
| 39 (39a) [NC40] | 3-{4-[7-(4-cyano-3-trifluoromethylphenyl)-8-oxo-6-thioxo-5,7-diazaspiro[3.4]oct-5-yl]-phenyl}-acrylic acid ethyl ester | n/a | 197 |
| 40 (40a) [NC41] | 4-{5-[4-(3-hydroxypropenyl)-phenyl]-8-oxo-6-thioxo-5,7-diazaspiro[3.4]oct-7-yl}-2-trifluoromethylbenzonitrile | n/a | 114 |
| 41-2 (41b) [NC42] | 3-{4-[7-(4-cyano-3-trifluoromethylphenyl)-8-oxo-6-thioxo-5,7-diazaspiro[3.4]oct-5-yl]-phenyl}-propionic acid methyl ester | No | n/a |
| 41-4 (41d) [NC43] | 3-{4-[7-(4-cyano-3-trifluoromethylphenyl)-8-oxo-6-thioxo-5,7-diaza-spiro[3.4]oct-5-yl]-phenyl}-propionamide | 224 | n/a |
| 41-5 (41e) [NC44] | 3-{4-[7-(4-Cyano-3-trifluoromethylphenyl)-8-oxo-6-thioxo-5,7-diaza-spiro[3.4]oct-5-yl]-phenyl}-N-methyl-propionamide | 234 | n/a |
| 41-6 (41f) [NC45] | 3-{4-[7-(4-cyano-3-trifluoromethylphenyl)-8-oxo-6-thioxo-5,7-diaza-spiro[3.4]oct-5-yl]-phenyl}-N-(2-hydroxyethyl)-propionamide | 732 | n/a |

| | | | |
|-------------------------|---|-----|-----|
| 42-2 (42b) [NC46] | 4-{4-[7-(4-cyano-3-trifluoromethylphenyl)-8-oxo-6-thioxo-5,7-diazaspiro[3.4]oct-5-yl]-phenyl}-butyric acid methyl ester | 432 | n/a |
| 42-4 (42d) [NC47] | 4-{4-[7-(4-Cyano-3-trifluoromethylphenyl)-8-oxo-6-thioxo-5,7-diaza-spiro[3.4]oct-5-yl]-phenyl}-butyramide | 112 | n/a |
| 42-5 (42e) [NC48] | 4-{4-[7-(4-Cyano-3-trifluoromethylphenyl)-8-oxo-6-thioxo-5,7-diaza-spiro[3.4]oct-5-yl]-phenyl}-N-methyl-butylamide | 92 | n/a |
| 43-4 (43e) [NC49] | 4-[8-Oxo-5-(4-piperazin-1-yl-phenyl)-6-thioxo-5,7-diazaspiro[3.4]oct-7-yl]-2-trifluoromethylbenzonitrile | 718 | n/a |
| 43-5 (43f) [NC50] | 4-{5-[4-(4-methanesulfonylpiperazin-1-yl)-phenyl]-8-oxo-6-thioxo-5,7-diazaspiro[3.4]oct-7-yl]-2-trifluoromethylbenzonitrile | 138 | n/a |
| 44-2 (44b) [NC51] | 44-2) 3-{4-[7-(4-Cyano-3-trifluoromethyl-phenyl)-8-oxo-6-thioxo-5,7-diaza-spiro[3.4]oct-5-yl]-phenyl}-acrylamide, | | 113 |

(*) No: the compound did not inhibit AR response reporter; (**) n/a: the compound was not examined in this assay.

5

Table 2

[0088] One previously unrecognized property of AR overexpression in hormone refractory prostate cancer is its ability to switch antagonists to agonists. Therefore, only those compounds with minimal or no agonistic activities are qualified to be anti-androgens for this disease. To determine agonistic activities of different compounds, we examined their stimulating activities on AR using the AR response reporter as the measure in the LN-AR system in the absence of R1881. Table 2 lists the agonistic activities of different compounds. Consistent with previous results, bicalutamide activated AR in hormone refractory prostate cancer. The diarylthiohydantoin derivatives such as examples 7-3b (NC7), 33 (NC34), 34 (NC35), and 35 (NC36) have no agonistic activity. In contrast, RU59063, and other anti-androgenic compounds listed as

10

examples in US Patent Number 5,705,654, such as examples 30-2, 30-3, 31-2, 31-3, and 24-3 (NC24-NC28) strongly activated AR in hormone refractory prostate cancer.

Agonistic activities of selective test substances on AR response reporter in hormone refractory prostate cancer

| Example | Name | Fold induction by increasing concentrations of compounds | | |
|------------------|---|--|-----------|------------|
| | | 0.1 μ M | 1 μ M | 10 μ M |
| DMSO | Dimethyl sulfoxide | 1.00 (*) | 1.00 | 1.00 |
| R1881 | methyltrienolone | 44.33 | n/a(**) | n/a |
| Bicalutamide | N-[4-cyano-3-(trifluoromethyl)phenyl]-3-[(4-fluorophenyl)sulfonyl]-2-hydroxy-2-methylpropanamide | 1.66 | 3.04 | 10.40 |
| 29 Comp. | 4-[3-(4-hydroxybutyl)-4,4-dimethyl-5-oxo-2-thioxoimidazolidin-1-yl]-2-trifluoromethylbenzonitrile | 10.99 | 20.84 | 34.62 |
| 7-3b (7c) [NC7] | 4-(8-oxo-6-thioxo-5-(4-methylphenyl)-5,7-diazaspiro[3.4]oct-7-yl)-2-trifluoromethylbenzonitrile | 0.87 | 1.19 | 0.89 |
| 33 (33a) [NC34] | 1-[3-(4-cyano-3-trifluoromethyl-phenyl)-5,5-dimethyl-2-thioxo-1-p-tolyl-imidazolidin-4-ylidene]-3-ethyl-thiourea | 1.30 | 1.18 | 1.28 |
| 34 (34a) [NC35] | 1-[7-(4-cyano-3-trifluoromethyl-phenyl)-6-thioxo-5-p-tolyl-5,7-diaza-spiro[3.4]oct-8-ylidene]-3-phenyl-thiourea | 1.19 | 1.41 | 1.17 |
| 35 (35a) [NC36] | 1-(4-Cyano-3-trifluoromethyl-phenyl)-3-[7-(4-cyano-3-trifluoromethyl-phenyl)-6-thioxo-5-p-tolyl-5,7-diaza-spiro[3.4]oct-8-ylidene]-thiourea | 1.26 | 1.10 | 1.30 |
| 30-2 Comp. (30b) | 4-(5-methyl-8-oxo-6-thioxo-5,7-diazaspiro[3.4]oct-7-yl)-2-trifluoromethylbenzonitrile | 14.88 | 19.41 | 35.22 |

| | | | | |
|----------------------------------|---|-------|-------|-------|
| [NC24] | | | | |
| 30-3 Comp. (30c) [NC25] | 4-(5-methyl-6,8-dioxo-5,7-diazaspiro[3.4]oct-7-yl)-2-trifluoromethylbenzonitrile | 11.39 | 14.26 | 30.63 |
| 31-2 Comp. (31b) [NC27] | 4-(1-methyl-4-oxo-2-thioxo-1,3-diazaspiro[4.4]non-3-yl)-2-trifluoromethylbenzonitrile | 17.03 | 16.63 | 33.77 |
| 31-3 Comp. (31c) [NC27] | 4-(1-methyl-2,4-dioxo-1,3-diazaspiro[4.4]non-3-yl)-2-trifluoromethylbenzonitrile | 11.99 | 19.77 | 38.95 |
| 24-3 Comp. (24c) [NC28] | 4-(4-oxo-2-thioxo-1,3-diazaspiro[4.4]non-3-yl)-2-trifluoromethylbenzonitrile | 14.88 | 22.48 | 37.09 |

(*) Fold induction: activities induced by a specific test substance over activities in DMSO vehicle; (**) n/a: the compound was not examined in this assay.

[0089] To examine the specificity of AR inhibitors, selective compounds were tested in LNCaP cells with an over expression of glucocorticoid receptor (GR), the closest member of AR in the nuclear receptor family. These cells also carry a GR response reporter and the reporter activity was induced by dexamethasone, a GR agonist and the induction was blocked by RU486, a GR inhibitor. Example 7-3b (NC7) (4-(8-oxo-6-thioxo-5-(4-methylphenyl)-5,7-diazaspiro[3.4]oct-7-yl)-2-trifluoromethyl benzonitrile) had no effect on GR in this system.

10 Effect of compounds on AR by measuring secreted levels of
prostate specific antigen (PSA)

[0090] It is well established that PSA levels are indicators of AR activities in prostate cancer. To

examine if the compounds affect AR function in a physiological environment, we determined secreted levels of endogenous PSA induced by R1881 in the AR-overexpressed LNCaP cells (LNCaP-AR, also abbreviated LN-AR). The LNCaP-AR cells are a line of lymph node carcinoma of prostate cells transduced with a plasmid that makes express androgen receptors. LNCaP-AR cells were maintained in Iscove's medium containing 10% FBS. Two days prior to drug treatment, the cells were grown in Iscove's medium containing 10% CS-FBS to deprive of androgens. The cells were split and grown in Iscove's medium containing 10% CS-FBS with appropriate concentrations of R1881 and the test compounds. After four days incubation, secreted PSA levels were assayed using PSA ELISA kits (American Qualex, San Clemente, CA)

[0091] The secreted PSA level of LNCaP-AR cells was strongly induced by 25 pM of R1881. In contrast, PSA was not induced in the parental LNCaP cells until concentration of R1881 reached 100 pM. This is consistent with our previous report that the AR in hormone refractory prostate cancer is hyper-sensitive to androgens. A dose-dependent inhibition on AR activity was carried out to determine the IC50s of different compounds in inhibiting PSA expression, and the results were listed in Table 1. IC50s of the selective compounds on PSA expression closely resemble those measured by the reporter assay, confirming that the diarylhydantoin derivatives are strong inhibitors of AR in hormone refractory prostate cancer.

[0092] We also examined agonistic activities of selective compounds on AR in hormone refractory prostate cancer using secreted PSA as the surrogate marker. To do this, androgen-starved AR over expressed LNCaP cells were incubated with increasing concentrations of the compounds for which a synthesis is described above in the absence of R1881 and secreted PSA in the culture medium was measured 4 days later.

[0093] Table 3 lists the agonistic activities of the selective compounds. Consistent with the results obtained from the reporter assay, the diarylthiohydantoin derivatives such as examples 7-3b (NC7), 33 (NC34), 34 (NC35), and 35 (NC36) have no agonistic activities. In contrast, RU59063, and other antiandrogenic compounds listed as examples in US patent no. 5,705,654, such as examples 30-2 (NC24), 30-3 (NC25), and 31-2 (NC26) stimulated PSA expression in hormone refractory prostate cancer.

Table 3

Agonistic activities of selective test substances on endogenous PSA in hormone refractory prostate cancer
Fold induction by increasing concentrations of compounds

| Example | Name | 0.1 μ M | 1 μ M | 10 μ M |
|---------|--------------------|-------------|-----------|------------|
| DMSO | Dimethyl sulfoxide | 1.00 (*) | 1.00 | 1.00 |
| R1881 | methyltrienolone | 20.69 | n/a(**) | n/a |

| | | | | |
|----------------------------------|---|------|-------|-------|
| Bicaluta mide | N-[4-cyano-3-(trifluoromethyl)phenyl]-3-[(4-fluorophenyl)sulfonyl]-2-hydroxy-2-methylpropanamide | 2.00 | 2.55 | 5.55 |
| 29 Comp. | 4-[3-(4-hydroxybutyl)-4,4-dimethyl-5-oxo-2-thioxoimidazolidin-1-yl]-2-trifluoromethylbenzonitrile | 6.88 | 11.50 | 21.50 |
| 7-3b (7c) [NC7] | 4-(8-oxo-6-thioxo-5-(4-methylphenyl)-5,7-diazaspiro[3.4]oct-7-yl)-2-trifluoromethylbenzonitrile | 1.25 | 1.20 | 1.15 |
| 33 (33a) [NC34] | 1-[3-(4-cyano-3-trifluoromethyl-phenyl)-5,5-dimethyl-2-thioxo-1-p-tolyl-imidazolidin-4-ylidene]-3-ethyl-thiourea | 1.06 | 1.30 | 0.85 |
| 34 (34a) [NC35] | 1-[7-(4-cyano-3-trifluoromethyl-phenyl)-6-thioxo-5-p-tolyl-5,7-diaza-spiro[3.4]oct-8-ylidene]-3-phenyl-thiourea | 1.31 | 1.05 | 0.90 |
| 35 (35a) [NC36] | 1-(4-Cyano-3-trifluoromethyl-phenyl)-3-[7-(4-cyano-3-trifluoromethyl-phenyl)-6-thioxo-5-p-tolyl-5,7-diaza-spiro[3.4]oct-8-ylidene]-thiourea | 1.44 | 1.30 | 1.05 |
| 30-2 Comp. (30b) [NC24] | 4-(5-methyl-8-oxo-6-thioxo-5,7-diazaspiro[3.4]oct-7-yl)-2-trifluoromethylbenzonitrile | 6.25 | 17.95 | 25.65 |
| 30-3 Comp. (30c) [NC25] | 4-(5-methyl-6,8-dioxo-5,7-diazaspiro[3.4]oct-7-yl)-2-trifluoromethylbenzonitrile | 7.50 | 15.20 | 23.75 |
| 31-2 Comp. (31b) [NC26] | 4-(1-methyl-4-oxo-2-thioxo-1,3-diazaspiro[4.4]non-3-yl)-2-trifluoromethylbenzonitrile | 8.13 | 18.20 | 17.50 |

(*) Fold induction: activities induced by a specific test substance over activities in DMSO vehicle; (**) n/a: the compound was not examined in this assay.

[0094]

Effect of compounds on AR mitochondrial activity by MTS assay

[0095] LNCaP-AR cells were maintained in Iscove's medium containing 10% FBS. The compounds were examined for their effect on growth of hormone refractory prostate cancer cells. Overexpressed LNCaP cells were used because these cells behave as hormone refractory prostate cancer cells in vitro and in vivo (1). We measured mitochondria activity by MTS assay, a surrogate for growth. LNCaP cells with overexpressed AR (LN-AR) were maintained in Iscove's medium containing 10% FBS. Two days prior to drug treatment, the cells were grown in Iscove's medium containing 10% CS-FBS to deprive of androgens. The cells were then split and grown in Iscove's medium containing 10% CS-FBS with appropriate concentrations of R1881 and increasing concentrations of the test compounds. After four days incubation, cell growth was monitored by MTS (Promega, Madison, WI).

[0096] Consistent with the reporter assay and PSA assay, growth of the AR-overexpressed LNCaP was stimulated by 25 microM of R1881, but the parental cells were not stimulated until R1881 concentration reached 100 microM. Figure 2 shows the inhibitory effect of selected compounds on growth of hormone refractory prostate cancer in the presence of 100 pM of R1881. The current clinical drug bicalutamide did not inhibit hormone refractory prostate cancer. In contrast, example 5-3b (NC2) (4-[3-(4-methylphenyl)-4,4-dimethyl-5-oxo-2-thioxoimidazolidin-1-yl]-2-trifluoromethyl-benzonitrile) and example 7-3b (NC7) (4-(8-oxo-6-thioxo-5-(4-methylphenyl)-5,7-diazaspiro[3.4]oct-7-yl)-2-trifluoromethylbenzonitrile) inhibited hormone refractory prostate cancer with high potency.

[0097] We examined if growth inhibition in the MTS assay occurs by targeting AR, example 5-3b (NC2) (4-[3-(4-methylphenyl)-4,4-dimethyl-5-oxo-2-thioxoimidazolidin-1-yl]-2-trifluoromethyl-benzonitrile) and example 7-3b (NC7) (4-(8-oxo-6-thioxo-5-(4-methylphenyl)-5,7-diazaspiro[3.4]oct-7-yl)-2-trifluoromethylbenzonitrile) were tested in DU-145 cells, a prostate cancer cell line that lacks AR expression. These compounds had no growth inhibitory effect on DU-145 cells. The compounds did not inhibit cells other than AR-expressed prostate cancer cells, as they had no growth effect on MCF7 and SkBr3, two commonly used breast cancer cells, or 3T3, a normal mouse fibroblast cell line.

[0098] Examples of in vitro biological activity of diarylthiohydantoin derivatives are shown in the Figures 3 and 4. For example, based on relative luciferase activity, Fig. 3 indicates that at a concentration of 500 nM the compounds ranked, in order of most active to least active as follows: NC67 > NC68 > NC66 > NC69 > NC77 = NC53 > bicalutamide. For example, based on relative PSA level, it was found that at a

concentration of 500 nM the compounds ranked, in order of most active to least active as follows: NC50 > NC48 > NC7 > NC43 > NC44 > NC49 > NC50 > NC45 > bicalutamide. For example, based on relative MTS units, Fig. 4 indicates that at a concentration of 500 nM the compounds ranked, in order of most active to least active as follows: NC70 > NC7 > NC122 > NC53 > bicalutamide.

5 **Inhibitory effect on hormone refractory and hormone sensitive prostate cancer xenograft tumors.**

[0099] Compounds of the invention are used to examine if the diarylhydantoin derivatives have in vivo effects on hormone refractory prostate cancer. First we examine this compound on xenograft tumors established from AR-overexpressed LNCaP cells. Engineered cells in Matrigel (Collaborative Biomedical) are injected subcutaneously into the flanks of the castrated male SCID mice. Tumor size is measured weekly in three dimensions using calipers. After xenograft tumors established (tumor size at least 40 mm³), mice with tumors are randomized and treated with different doses of compounds orally once daily. The inhibitory effect on growth of AR-overexpressed LNCaP xenograft model is studied as follows. Mice with established LN-AR xenograft tumors are randomized and treated with indicated compounds orally once daily. Tumor size are measured by caliber.

[00100] Consistent with clinical observation, current clinical drug bicalutamide did not inhibit growth of hormone refractory prostate cancer (same as vehicle). In contrast, compounds according to the invention inhibit growth of these tumors and the inhibition is dose-dependent. Furthermore, the compounds inhibit PSA expression, the clinical marker for hormone refractory prostate cancer.

20 [00101] Compounds of the invention are also tested in another xenograft model of hormone refractory prostate cancer, hormone refractory LAPC4. This model was established from passaging of hormone sensitive prostate cancer in castrated mice, which mimics the clinical progression of prostate cancer (2). Similar to the finding using AR-overexpressed LNCaP xenograft model, current clinical drug bicalutamide did not inhibit growth and PSA expression in hormone refractory LAPC4 xenograft model (same as vehicle). In contrast, compounds of the invention inhibited growth and PSA expression of these tumors.

[00102] Figure 6 presents the results of an experiment in which cells from the LNCaP hormone sensitive model were xenografted into mice (10⁶ cells of LNCaP were injected into mice). A first set of mice was treated with NC53, a second set of mice was treated with Casodex, and a third set of mice was treated with vehicle solution. Each set included 6 mice. The mice were treated with 10 mg/kg per day. Fig. 6

presents the results as a graph of tumor volume as a function of time. Mice treated with vehicle solution as a control exhibited the most rapid increase in tumor volume. Mice treated with Casodex and mice treated with NC53 exhibited similar rates of tumor growth, slower than mice treated with vehicle solution.

Inhibitory effect on growth of hormone sensitive prostate cancer cells.

5 [00103] To determine if the diarylthiahydantoin derivatives also inhibit hormone sensitive prostate cancer cells, we test some selective compounds on growth of LNCaP cells by measuring MTS of mitochondria activities. Androgen starved LNCaP cells are treated with increasing concentrations of DMSO as vehicle or test substances in the presence of 1 pM of R1881. After 4 days of incubation, cell growth is measured by MTS assay. Compounds of the invention inhibit hormone sensitive prostate cancer with a
10 higher potency than bicalutamide.

In vivo biological assay

[00104] All animal experiments were performed in compliance with the guidelines of the Animal Research Committee of the University of California at Los Angeles. Animals were bought from Taconic and maintained in a laminar flow tower in a defined flora colony. LNCaP-AR and LNCaP-vector cells were
15 maintained in RPMI medium supplemented with 10% FBS. 10^6 cells in 100 μ l of 1:1 Matrigel to RPMI medium were injected subcutaneously into the flanks of intact or castrated male SCID mice. Tumor size was measured weekly in three dimensions (length x width x depth) using calipers. Mice were randomized to treatment groups when tumor size reached approximately 100 mm³. Drugs were given orally every day at 10 mg/kg and 50 mg/kg. To obtain pharmacodynamic readout, the animals were imaged via an optical CCD
20 camera, 3 hours after last dose of the treatment. A ROI is drawn over the tumor for luciferase activity measurement in photon/second. The right panels were a representation of the ROIs measurements. Data are shown in Figures 7 and 8. Over 18 days NC53 was effective to prevent tumor growth and even to cause tumor shrinkage, and was distinctly more effective than bicalutamide.

[00105] The pharmacokinetics of bicalutamide, 4-[7-(4-cyano-3-trifluoromethylphenyl)-8-oxo-6-thioxo-5,7-diaza-spiro[3.4]oct-5-yl]-toluene [NC7], *N*-methyl-4-{4-[7-(4-cyano-3-trifluoromethylphenyl)-8-oxo-6-thioxo-5,7-diaza-spiro[3.4]oct-5-yl]phenyl}butanamide [NC48], and *N*-methyl-4-[7-(4-cyano-3-trifluoromethylphenyl)-8-oxo-6-thioxo-5,7-diaza-spiro[3.4]oct-5-yl]-2-fluorobenzamide (52d) [NC53] were
25 evaluated in vivo using 8 week-old FVB mice which were purchased from Charles River Laboratories. Mice were divided into groups of three for each time points. Two mice were not treated with drug and two other
30 mice were treated with vehicle solution. Each group was treated with 10 mg per kilogram of body weight.

[00106] The drug was dissolved in a mixture 1:5:14 of DMSO : PEG400 : H₂O. (Vehicle solution) and was administered into mice through the tail vein. The animals are warmed under a heat lamp for approximately 20 minutes prior to treatment to dilate their tail vein. Each mouse was placed into a mouse restrainer (Fisher Sci. Cat# 01-288-32A) and was injected with 200 µl of drug in vehicle solution into the dilated tail vein. After drug administration, the animals were euthanized via CO₂ inhalation at different timepoints: 5 mn, 30 mn, 2 h, 6 h, 16 h. Animals were immediately bleed after exposure to CO₂ via cardiac puncture (1 ml BD syringe + 27G 5/8 needle). For oral dosage, the drug was dissolved in a mixture 50:10:1:989 of DMSO : Carboxymethylcellulose : Tween80:H₂O before oral administration via a feeding syringe.

[00107] The serum samples were analyzed to determine the drug's concentration by the HPLC which (Waters 600 pump, Waters 600 controller and Waters 2487 detector) was equipped with an Alltima C18 column (3µ, 150 mm×4.6 mm). The NC7, NC48, and NC53 compounds were detected at 254 nm wave length and bicalutamide was detected at 270 nm wave length.

[00108] The samples for HPLC analysis were prepared according to the following procedure:

- Blood cells were separated from serum by centrifugation.

- To 400 µl of serum were added 80 µl of a 10 µM solution of an internal standard and 520 µl of acetonitrile. Precipitation occurred.

- The mixture was vortexed for 3 minutes and then placed under ultrasound for 30 minutes.

- The solid particles were filtered off or were separated by centrifugation.

- The filtrate was dried under an argon flow to dryness. The sample was reconstructed to 80 µl with acetonitrile before analyzing by HPLC to determine the drug concentration.

- Standard curve of drug was used to improve accuracy.

[00109] The concentration of NC53 in plasma as a function of time resulting from intravenous and from oral administration is shown in figure 9. The steady state concentration (C_{ss}) of bicalutamide, NC48, and NC53 is shown in Table 4. The concentration at steady state of NC53 is essentially as good as that of bicalutamide, and substantially better than NC48.

| Name | IC50 [nM] | LogP | Css,10 mg/kg [μM] | Css,25 mg/kg [μM] | Css,50 mg/kg [μM] |
|-------------|-----------|------|----------------------|----------------------|----------------------|
| Bic. | 1000 | 2.91 | 10.0 | 11.4 | 11.9 |
| NC48 | 92 | 3.44 | 0.39 | 0.43 | 0.40 |
| NC53 | 122 | 3.20 | 9.9 | 10.7 | 10.2 |

Table 4. Steady-state concentration of bicalutamide, NC48, and NC53 in mice plasma.

[00110] The androgen receptor activity can encompass several aspects of stimulation and of inhibition of androgen receptor behavior, including, but not limited to, the following: inhibitory concentration (IC50) in an AR response reporter system or a prostate specific antigen secreting system; fold induction associated with increasing concentrations in an AR response reporter system or a prostate specific antigen secreting system; associated tumor growth in an animal; the binding affinity of an androgen receptor to a compound; androgen receptor recruitment to a prostate specific antigen enhancer or a prostate specific antigen promoter; androgen receptor nuclear translocation; and destabilization of an androgen receptor protein.

In vitro assays

[00111] Figure 10 presents the relative binding affinities of compounds for the ligand binding domains of rat androgen receptor (rat AR) as determined with competitor assay kits (Invitrogen). Fluorescence polarization was used as a read-out. Each hormone dose was performed in triplicate and the relative error was determined by calculating the standard error of the three values from the mean. The study controlled for minimal competition (vehicle alone), no receptor, no fluorescent ligand, and maximal competition (10^{-5} M R1881, progesterone, E2 or dexamethasone). The curves were fit using a single binding site competition model (the Prism statistical analysis software package was used. R1881 had the lowest equilibrium dissociation constant, $K_i = 4$ nM (and thus the rat androgen receptor had the highest affinity for R1881 of the four compounds tested). RU59063 had an equilibrium dissociation constant of $K_i = 20$ nM, and NC53 had an equilibrium dissociation constant of $K_i = 0.8$ μM. Casodex had an equilibrium dissociation constant of $K_i = 0.4$ μM (and thus the rat androgen receptor had the lowest affinity for Casodex of the four compounds tested). NC53 and Casodex had similar equilibrium dissociation constants, and, thus, rat androgen receptor had a similar affinity for these compounds.

[00112] NC53 prevented androgen receptor (AR) recruitment and RNA polymerase II (Pol II)

recruitment to PSA enhancer and to PSA promoter. Figure 11 presents the results of the study. Materials used were Chromatin IP with AR (Upstate, cat# 06-680) and Pol II (Covance, cat# MMS-126R). LNCaP (ATCC) cells were plated in full serum. On the day of the experiment, the plate was washed once with 1x PBS and 5% CSS was added for 3 days. For a first set of experiments 10 uM of NC53 was added (R), for a second set of experiments 10 uM of bicalutamide (C) was added, and for a third set of experiments 1 nM of R1881 was added (+). Each of these compounds was added for 6 hours. In a fourth set of experiments, a control, no additional compound was added (-). 6 hours timepoint was run at 28 cycles. ChIP kits from Upstate (cat# 17-295) were used. Enhancer and promoter primers were obtained from Louie (PNAS 2003 Vol. 100, pp. 2226-2230) and Shang (Molecular Cell 2002 vol. 9, pp. 601-610), respectively. The darker image for experiments in which NC53 (R) was added indicated that NC53 prevented androgen receptor and prevented RNA polymerase II from forming a transcription complex on the prostate specific antigen (PSA) gene. By contrast, the lighter image for experiments in which bicalutamide (Casodex, C) was added indicated that in the presence of bicalutamide androgen receptor and RNA polymerase II were still recruited to the PSA elements to transcribe PSA mRNA.

[00113] NC53 inhibited androgen receptor nuclear translocation in LNCaP cells. Figures 12 and 13 present the results of the study. LNCaP cells were plated in 5% CSS. A first set of cells was treated with 10 uM NC53 (R), a second set of cells was treated with 10 uM bicalutamide (C), and a third set of cells was treated with 1 nM R1881 (+). A fourth set of cells served as a control (-). TOPO I (Santa Cruz, cat# sc-32736) was used to control for nuclear fraction, and GAPDH (Santa Cruz, cat# sc-20357) was used to control for cytoplasmic fraction. LNCaP cells were harvested for subcellular fractionation or stained with a FITC (Santa Cruz) labeled antibody against androgen receptor (AR) (Santa Cruz, cat# sc-815). From the subcellular fractionation, images were obtained, as shown in Fig. 12. The darker image in the nuclear fraction for the bicalutamide (Casodex, C) treated sample indicated that bicalutamide induced androgen receptor nuclear translocation. The light image for the NC53 (R) treated sample indicated that NC53 abrogated nuclear translocation. For the AR-FITC assay, cover slips were mounted on glass slides using DAPI-containing medium, and cells were imaged 24 hours later using a fluorescence Nikon microscope at X60 with filters for DAPI and FITC. In the AR-FITC assay, the nuclei of the R1881 and of the bicalutamide treated cells were distinctly green, as shown in Fig. 13 indicating that nuclear translocation of the androgen receptor occurred. By contrast, the nuclei of the DMSO and of the NC53 treated cells were less green.

[00114] NC53 destabilized androgen receptor proteins in LNCaP cells. Figure 14 shows the results of the study. The study was conducted by plating 10^5 LNCaP (fgc) cells in 5% CSS for 3 days. 100 pM of

R1881 was added to a first set of cells (+), 10 uM of bicalutamide was added to a second set of cells (B), 10 uM of NC53 was added to a third set of cells (RD), 100 pM of R1881 and 10 uM of bicalutamide was added to a fourth set of cells (B+), and 100 pM of R1881 and 10 uM of NC53 was added to a fifth set of cells (RD+). Neither R1881, bicalutamide, nor NC53 was added to a sixth set of cells (-). The cells were allowed to reside with the added bicalutamide, NC53, and/or R1881 for 24 hours (or in the case of the (-) set, without any of these for 24 hours). In Fig. 14, the dark image for the set to which bicalutamide (B) was added and for the set to which bicalutamide and R1881 (B+) were added indicated that the androgen receptor protein was level when these combinations of compounds were added. By contrast, the light image for the set to which NC53 (RD) was added and for the set to which NC53 and R1881 (RD+) were added indicated that the addition of NC53 resulted in the degradation of androgen receptor proteins, whether or not R1881 was present.

Ranking of Compounds in Tiers

[00115] Tables 5 – 10 present diarylhydantoin compounds grouped into Tiers 1-6. Table 11 presents diarylhydantoin compounds which have not been placed into a tier. The placement of compounds into tiers was based on available data coupled with analytical judgment. Data considered included in vitro assays (AR response reporter system in LNCaP cell line, PSA level measurement, MTS mitochondrial assay) and in vivo experiments (tumor size measured directly or by emission induced by luciferase reporter gene, pharmacokinetic assays based on blood plasma levels). Not every compound was subjected to each assay. Not all data that was generated is shown. Judgment was applied in ranking compounds relative to each other for their utility in treating prostate cancer, in particular when ranking two compounds for which the same experiments were not performed. Characteristics considered in establishing the ranking include AR antagonism activity, lack of AR agonism in hormone refractory cells, prevention of tumor growth, tumor shrinkage, and pharmacokinetic behavior, with a longer residence time in blood being advantageous.

25 Tier 1

[00116] Generally, Tier 1 compounds are diarylthiohydantoins with a disubstituted left hand aryl ring that are disubstituted on the right hydantoin carbon, and have either an oxygen or N substituent on the left hydantoin carbon. It is expected that the amido substituent hydrolyzes to an oxygen in aqueous solutions such as encountered in biological systems, in vitro and in vivo. NC63 has good activity with an iodine instead of a CF₃ substituent on the left hand aryl ring.

[00117] Tier 1 compounds (see Table 5) were judged to be much better than bicalutamide for treating prostate cancer. However, NC7 and NC48 were found to metabolize fast, that is, have a short residence time in blood. NC53 had desirable pharmacokinetics.

[00118] Figure 16 shows that under treatment with bicalutamide, PSA levels for LNCaP cells stayed the same or increased relative to treatment with vehicle solution, whereas under treatment with NC53, PSA levels decreased. Figure 17 illustrates that under treatment with vehicle solution, tumors continued to increase in size. By contrast, under treatment with NC53 at a dose of 1 mg per kg body weight per day, the rate of tumor increase decreased, and the size of the tumor appeared to be stabilizing after about 17 days. Under treatment with NC53 at a dose of 10 mg per kg body weight per day, tumor size decreased with time. Figure 18 illustrates that under treatment with NC53 at a dose of 10 mg per kg body weight per day, photon emission associated with luciferase activity decreased. Figure 19 shows that treatment with NC53 at this dose resulted in a decrease or stabilization of tumor size and a decrease in photon emission associated with luciferase activity.

[00119] Figure 20 shows that under treatment with NC53, NC54, NC55, NC56, and NC57 at doses of 100, 200, 500, and 1000 nM, PSA levels of LN-AR cells decreased. Moreover, the higher the dose, the lower the PSA level. Figure 22 presents urogenital tract weight and rate of photon emission associated with luciferase activity initially and after 14 days of treatment with bicalutamide or with NC53 for intact and castrated mice. The weight and rate of photon emission increased for both intact and castrated mice. Treatment of castrated mice with NC53 resulted in a decrease in weight and photon emission with respect to the untreated castrated mice, as did treatment with bicalutamide.

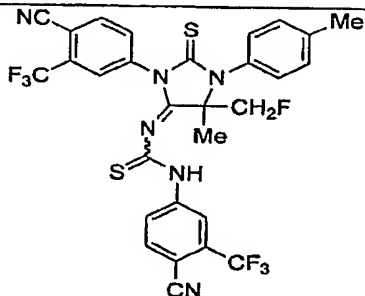
[00120] Thus, Tier 1 compounds are particularly advantageous for use as AR antagonists, and as therapeutic agents for hormone refractory prostate cancer. They may be useful to treat other AR related diseases or conditions such as benign prostate hyperplasia, hair loss, and acne. These and related compounds may also be useful as modulators of other nuclear receptors, such as glucocorticoid receptor, estrogen receptor, and peroxisome proliferator-activated receptor, and as therapeutic agents for diseases in which nuclear receptors play a role, such as breast cancer, ovarian cancer, diabetes, cardiac diseases, and metabolism related diseases. They may be useful in assays e.g. as standards, or as intermediates or prodrugs.

TABLE 5

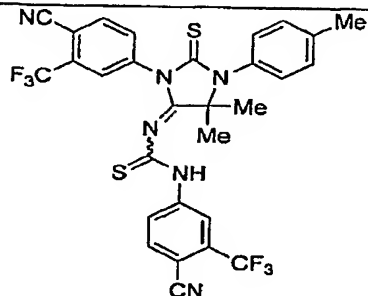
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|------------------|--|
| NC2 | |
| NC3 | |
| NC1 | |
| NC6 | |
| NC58 | |
| NC7 | |
| NC45 | |
| NC16 | |
| NC33 | |
| NC34 | |
| NC35 | |
| NC36 | |

TIER 1 COMPOUNDS

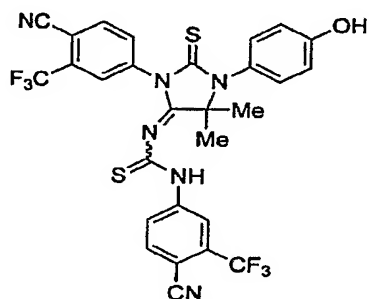
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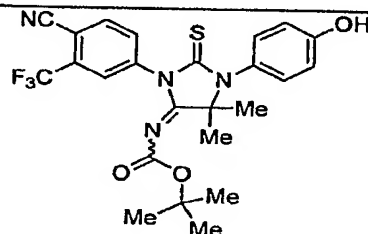
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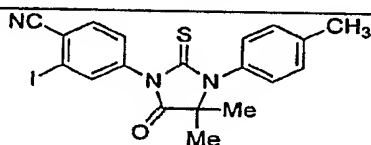
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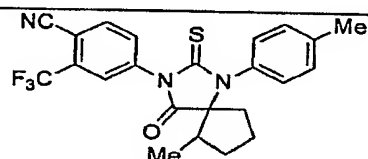
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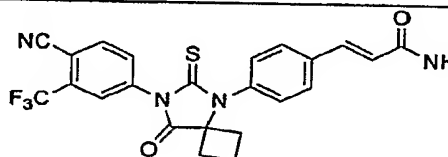
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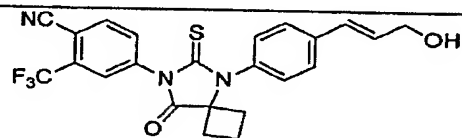
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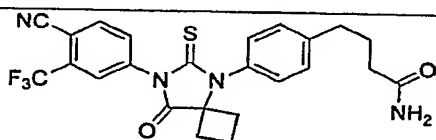
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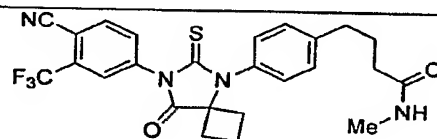
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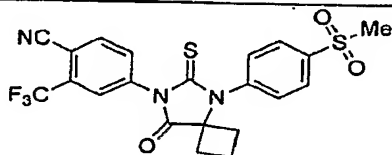
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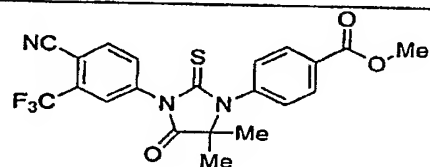
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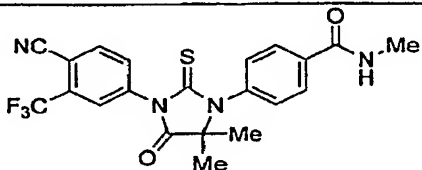
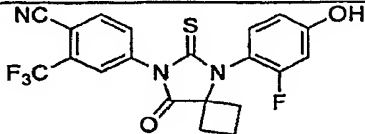
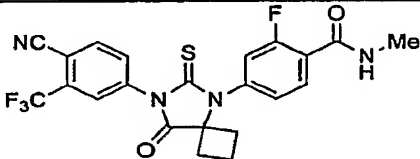
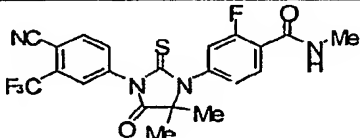
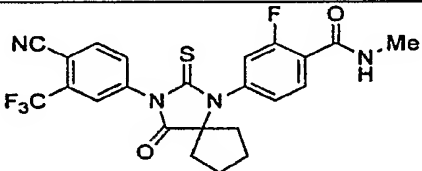
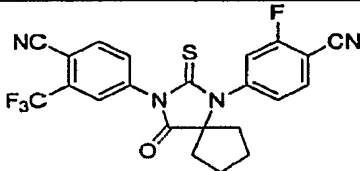
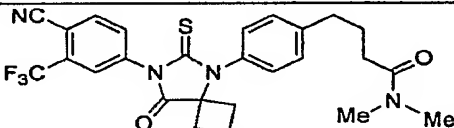
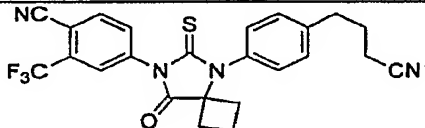
NC66



NC67



TIER 1 COMPOUNDS

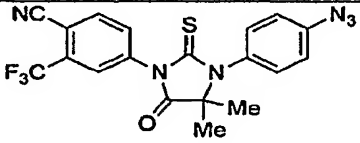
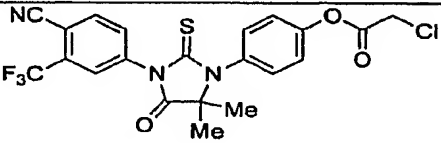
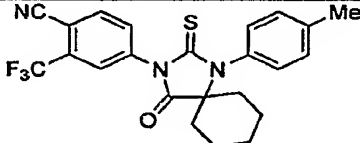
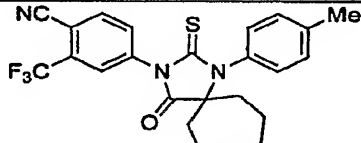
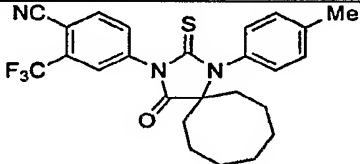
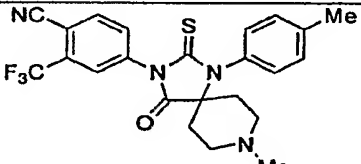
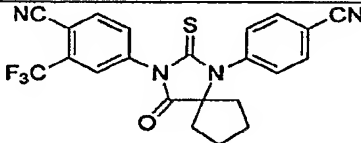
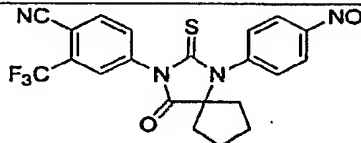
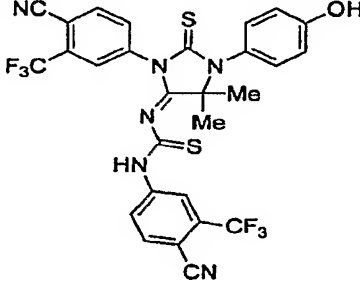
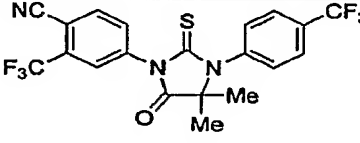
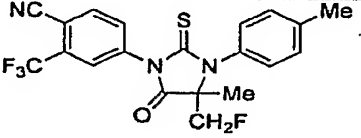
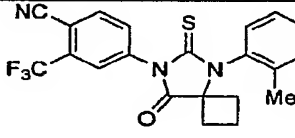
| | |
|--|---|
| <i>NC 68</i>  | <i>NC 69</i>  |
| <i>NC 53</i>  | <i>NC 54</i>  |
| <i>NC 55</i>  | <i>NC 70</i>  |
| <i>NC 56</i>  | <i>NC 57</i>  |

Tier 2

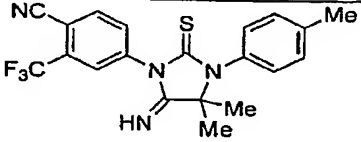
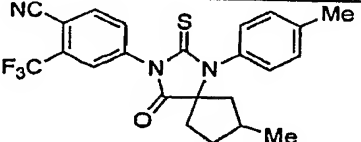
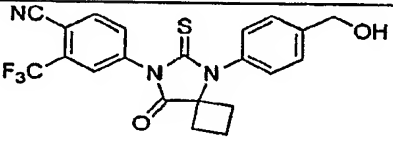
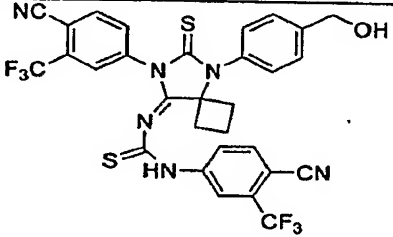
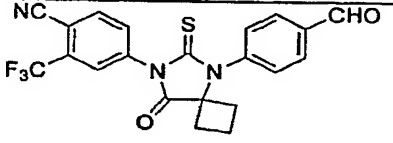
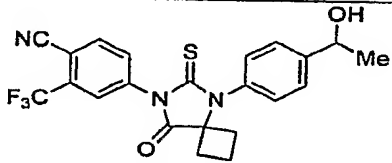
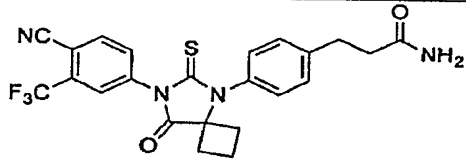
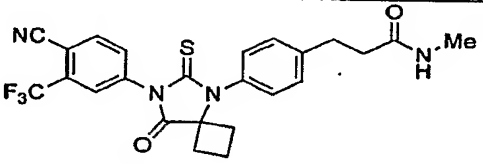
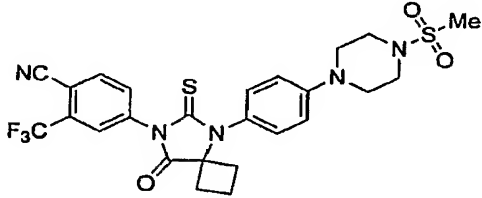
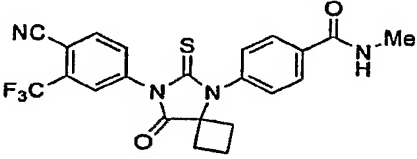
[00121] Tier 2 compounds (see Table 6) were significantly better than bicalutamide for treating prostate cancer, although there were indications that NC12 could act as an agonist. Figure 3 illustrates that compounds NC66, NC67, NC68, NC53, and NC69 in Tier 1 and NC77 in Tier 2 dosed at concentrations ranging from 125 nM to 1000 nM acted to reduce luciferase activity in LNCaP-AR cells whereas control solutions of DMSO and of bicalutamide had little or no effect. It was found that at concentrations of 1000 nM, compounds NC7 and NC48, in Tier 1, caused a greater decrease in PSA level of LNCaP-AR cells than NC43, NC44, and NC50 in Tier 2. Figure 7 presents tumor volume over time, and illustrates that under treatment with bicalutamide or vehicle solution, tumors continued to grow, whereas under treatment with NC53, in Tier 1, tumors decreased in size. Figure 8 illustrates that photon emission associated with luciferase activity remained about the same or increased under treatment with bicalutamide relative to treatment with vehicle solution, whereas photon emission decreased under treatment with NC53. Figure 23 illustrates that under treatment with bicalutamide, there was little or no decrease in PSA levels, whereas under treatment with NC48 and NC53, PSA levels decreased. Figure 24 illustrates that the IC_{50} for NC7, NC48, and NC53, in Tier 1, was much lower than the IC_{50} for bicalutamide.

[00122] Generally, Tier 2 compounds are structurally similar to Tier 1 compounds, but with different substituents on the right hand aryl ring. Tier 2 compounds are advantageous for use as AR antagonists, and as therapeutic agents for hormone refractory prostate cancer. They may be useful to treat other AR related diseases or conditions such as benign prostate hyperplasia, hair loss, and acne. These and related compounds may also be useful as modulators of other nuclear receptors, such as estrogen receptor and peroxisome proliferator-activated receptor, and as therapeutic agents for diseases in which nuclear receptors play a role, such as breast cancer, ovarian cancer, diabetes, cardiac diseases, and metabolism related diseases. They may be useful in assays e.g. as standards, or as intermediates or prodrugs.

TABLE 6

| TIER 2 COMPOUNDS | |
|--|--|
| <p><i>NC 71</i></p>  <p>(comparative)</p> | <p><i>NC 5</i></p>  |
| <p><i>NC 8</i></p>  | <p><i>NC 9</i></p>  |
| <p><i>NC 72</i></p>  | <p><i>NC 11</i></p>  |
| <p><i>NC 12</i></p>  | <p><i>NC 13</i></p>  |
| <p><i>NC 73</i></p>  | <p><i>NC 20</i></p>  |
| <p><i>NC 22</i></p>  | <p><i>NC 23</i></p>  |

TIER 2 COMPOUNDS

| | |
|---|--|
| <i>NC 74</i>  | <i>NC 75</i>  |
| <i>NC 37</i>  | <i>NC 76</i>  |
| <i>NC 38</i>  | <i>NC 39</i>  |
| <i>NC 43</i>  | <i>NC 44</i>  |
| <i>NC 50</i>  | <i>NC 77</i>  |

Tier 3

[00123] Tier 3 compounds (see Table 7) were judged to be slightly better than bicalutamide for treating prostate cancer. NC43, NC44, and NC50 (in Tier 2) caused a greater decrease in PSA level of LNCaP-AR cells than NC45 and NC49, in Tier 3. All of these compounds caused a greater decrease in PSA
5 level than bicalutamide.

[00124] Other Tier 3 compounds (not shown) were not diarylthiohydantoin, and were comparable in activity to prior art monoarylhydantoin compounds NC83, NC79, and NC80.

[00125] Thus, Tier 3 compounds are useful as AR antagonists, and as therapeutic agents for hormone refractory prostate cancer. They may be useful to treat other AR related diseases or conditions such
10 as benign prostate hyperplasia, hair loss, and acne. These and related compounds may also be useful as modulators of other nuclear receptors, such as estrogen receptor and peroxisome proliferator-activated receptor, and as therapeutic agents for diseases in which nuclear receptors play a role, such as breast cancer, ovarian cancer, diabetes, cardiac diseases, and metabolism related diseases. They may be useful in assays e.g. as standards, or as intermediates or prodrugs.

15

TABLE 7

| TIER 3 COMPOUNDS | |
|---|---|
| <p><i>NC78</i></p> <p>(comparative)</p> | <p><i>NC79</i></p> <p>(comparative)</p> |
| <p><i>NC80</i></p> <p>(comparative)</p> | <p><i>NC81</i></p> <p></p> |
| <p><i>NC82</i></p> <p></p> | <p><i>NC42</i></p> <p></p> |
| <p><i>NC46</i></p> <p></p> | <p><i>NC45</i></p> <p></p> |
| <p><i>NC49</i></p> <p></p> | |

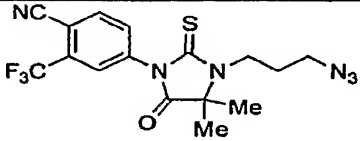
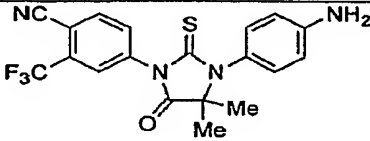
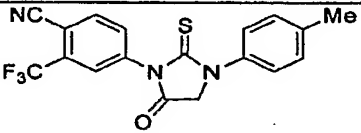
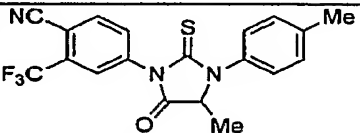
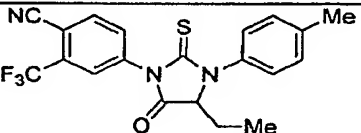
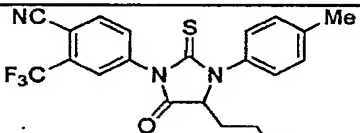
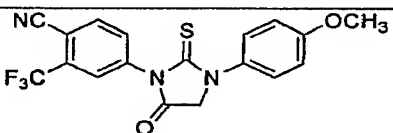
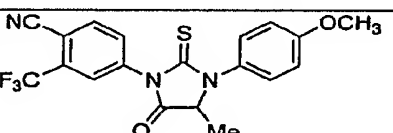
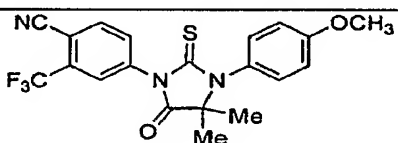
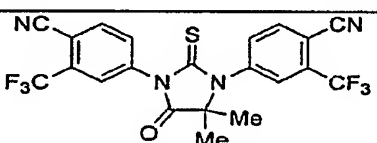
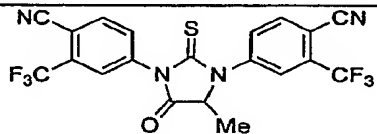
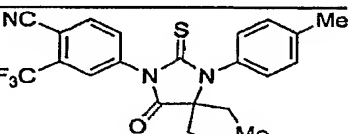
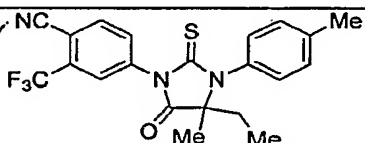
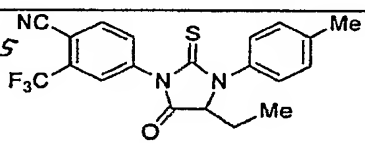
Tier 4

[00126] Tier 4 compounds (see Table 8) were judged to be no better than bicalutamide for treating prostate cancer. Tier 4 NC93 and NC94 and Tier 1 NC7, for example, differ only in the substituent on the lower right carbon of the hydantoin ring. The substituents on the right hand aryl ring may also affect activity.

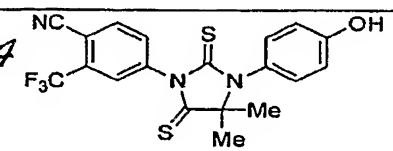
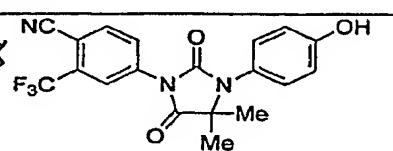
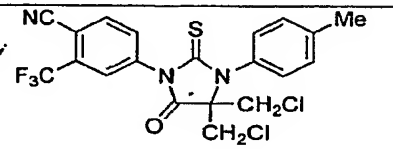
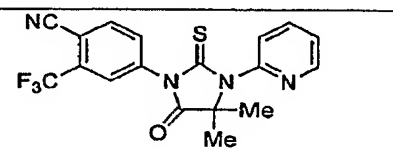
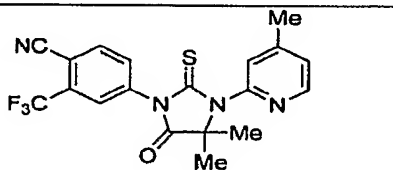
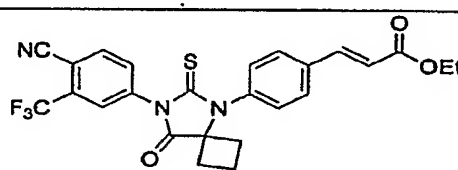
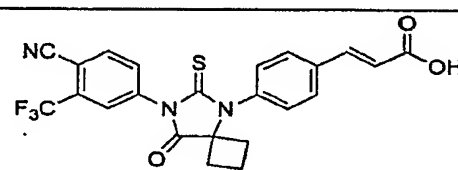
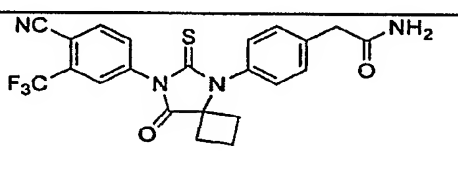
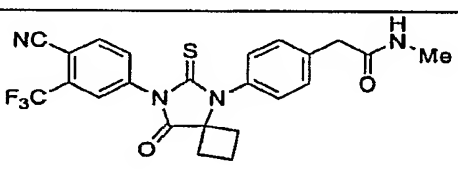
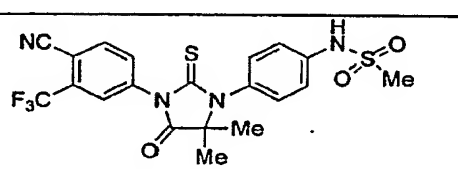
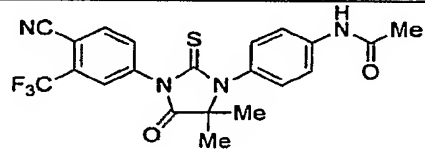
[00127] Some Tier 4 compounds (including those shown and others that are not shown) were not diaryl compounds (lacking the right hand aryl ring), were not thiohydantoins, were not disubstituted on the carbon on the lower right hand of the hydantoin ring, and/or had substituents other than oxygen or amido on the lower left hand carbon of the hydantoin ring. This provides evidence of the surprising advantages of diarylthiohydantoins that are disubstituted on the lower right hand carbon of the hydantoin ring and have oxygen or amido on the lower left hand carbon of the hydantoin ring.

Thus, Tier 4 compounds may be useful as AR antagonists, and as therapeutic agents for hormone refractory prostate cancer, at least to the extent that they are comparable to bicalutamide. They may be useful to treat other AR related diseases or conditions such as benign prostate hyperplasia, hair loss, and acne. These and related compounds may also be useful as modulators of other nuclear receptors, such as estrogen receptor and peroxisome proliferator-activated receptor, and as therapeutic agents for diseases in which nuclear receptors play a role, such as breast cancer, ovarian cancer, diabetes, cardiac diseases, and metabolism related diseases. They may be useful in assays e.g. as standards, or as intermediates or prodrugs.

TABLE 8

| TIER 4 COMPOUNDS | |
|--|--|
| <p><i>NC 83</i></p>  <p>(comparative)</p> | <p><i>NC 4</i></p>  |
| <p><i>NC 84</i></p>  | <p><i>NC 85</i></p>  |
| <p><i>NC 86</i></p>  | <p><i>NC 87</i></p>  |
| <p><i>NC 88</i></p>  | <p><i>NC 89</i></p>  |
| <p><i>NC 90</i></p>  | <p><i>NC 91</i></p>  |
| <p><i>NC 92</i></p>  | <p><i>NC 93</i></p>  |
| <p><i>NC 94</i></p>  | <p><i>NC 95</i></p>  |

TIER 4 COMPOUNDS

| | |
|--|--|
| <i>NC 14</i>  | <i>NC 18</i>  |
| <i>NC 21</i>  | <i>NC 29</i>  |
| <i>NC 30</i>  | <i>NC 40</i>  |
| <i>NC 96</i>  | <i>NC 97</i>  |
| <i>NC 98</i>  | <i>NC 99</i>  |
| <i>NC 100</i>  | |

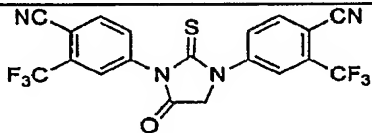
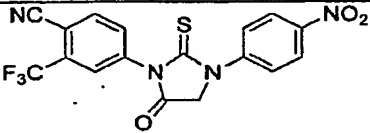
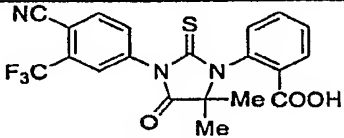
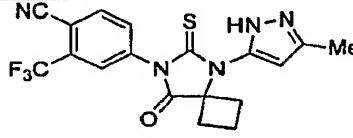
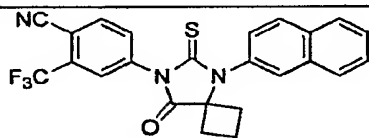
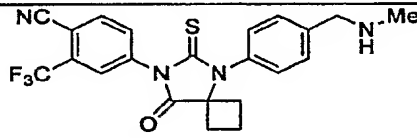
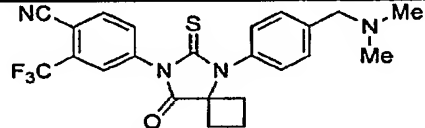
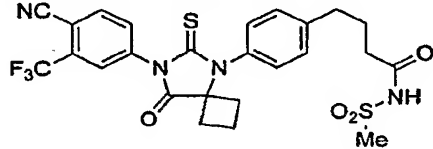
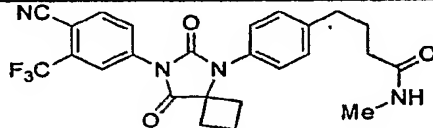
Tier 5

[00128] Tier 5 compounds (see Table 9) were inactive or nearly inactive, and thus, were worse than bicalutamide for treating prostate cancer. The substituents on the right hand aryl ring are important to determining activity.

5 [00129] Some Tier 5 compounds (some of which are shown and some that are not shown) were not diaryl compounds (lacking the right hand aryl ring), were not thiohydantoin, were not disubstituted on the carbon on the lower right hand of the hydantoin ring, and/or had substituents other than oxygen or amido on the lower left hand carbon of the hydantoin ring. This provides evidence of the surprising advantages of diarylthiohydantoins that are disubstituted on the lower right hand carbon of the hydantoin ring and have
10 oxygen or amido on the lower left hand carbon of the hydantoin ring. In particular, the terminal substituent in NC103, NC104, and NC106 ($\text{CH}_2\text{NR}_x\text{R}_y$, where $\text{R}_{x,y} = \text{H}$ or methyl) is not seen as contributing to activity in these compounds.

[00130] Tier 5 compounds would not be desirable for treatment of prostate cancer or as AR antagonists, although these and related compounds may be useful as modulators of other nuclear receptors,
15 such as estrogen receptor and peroxisome proliferator-activated receptor, and as therapeutic agents for diseases in which nuclear receptors play a role, such as breast cancer, ovarian cancer, diabetes, cardiac diseases, and metabolism related diseases. They may be useful in assays e.g. as standards, or as intermediates or prodrugs.

TABLE 9

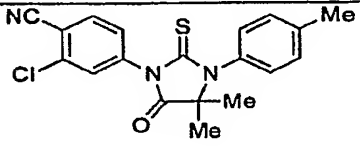
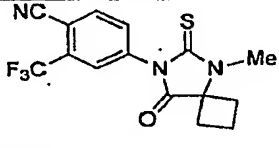
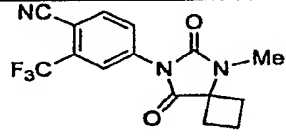
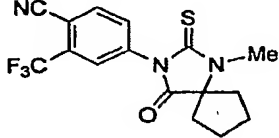
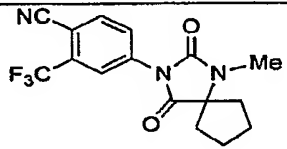
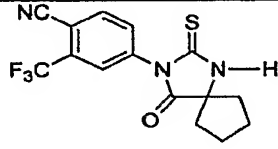
| TIER 5 COMPOUNDS | |
|--|--|
| <i>NC 101</i>  | <i>NC 102</i>  |
| <i>NC 19</i>  | <i>NC 31</i>  |
| <i>NC 32</i>  | <i>NC 103</i>  |
| <i>NC 104</i>  | <i>NC 105</i>  |
| <i>NC 106</i>  | |

Tier 6

[00131] Tier 6 compounds (see Table 10) were inactive or nearly inactive, and furthermore were strong agonists, and thus were much worse than bicalutamide for treating prostate cancer. The comparative compounds ranked very poor relative to the inventive compounds. Notably, NC107 had very poor activity, with a chlorine substituent on the left hand aryl ring, whereas NC2, with a trifluoromethane, and NC63, with iodine, ranked in Tier 1. The results for the Tier 6 compounds provide evidence of the surprising advantages of diarylthiohydantoin compounds that are disubstituted on the lower right hand carbon of the hydantoin ring and have oxygen or amido on the lower left hand carbon of the hydantoin ring, and have certain substituents on the left hand aryl ring.

10 [00132] Tier 6 compounds would not be desirable for treatment of prostate cancer or as AR antagonists.

TABLE 10

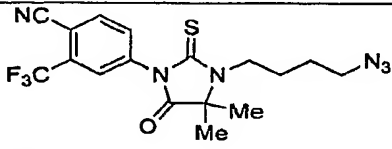
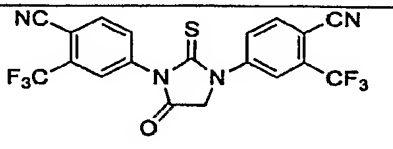
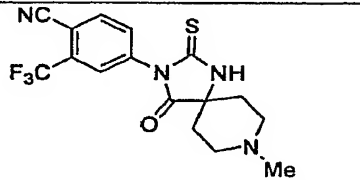
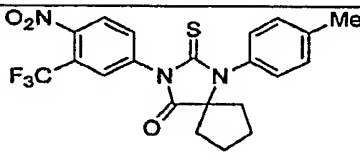
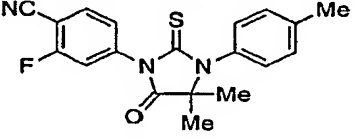
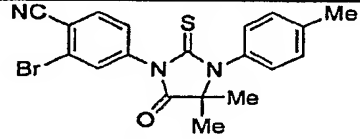
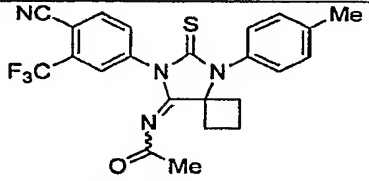
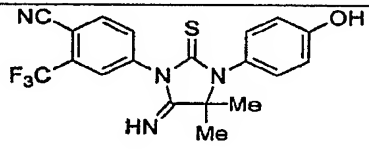
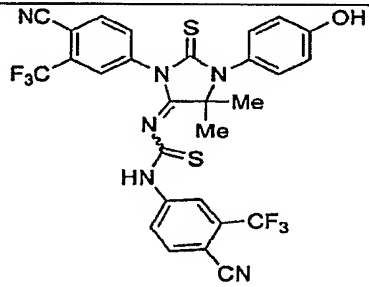
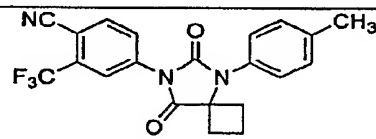
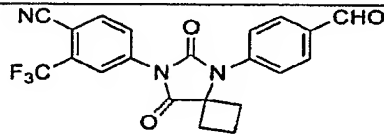
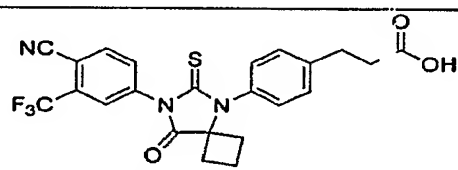
| TIER 6 COMPOUNDS | |
|--|---|
| <p><i>NC 107</i></p>  | <p><i>NC 24</i></p>  <p>(comparative)</p> |
| <p><i>NC 25</i></p>  <p>(comparative)</p> | <p><i>NC 26</i></p>  |
| <p><i>NC 27</i></p>  <p>(comparative)</p> | <p><i>NC 28</i></p>  |

Untiered compounds

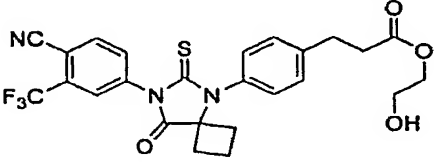
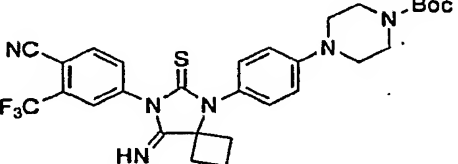
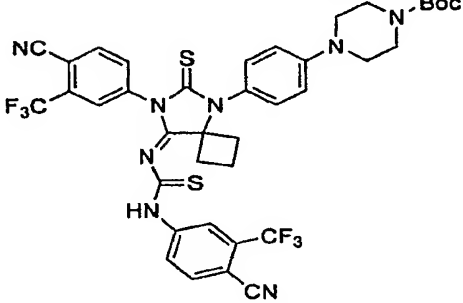
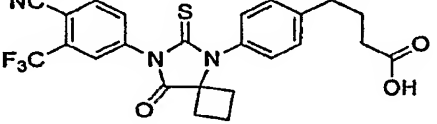
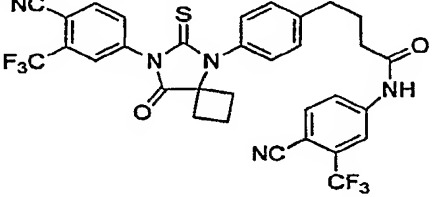
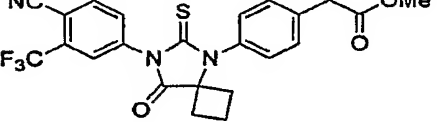
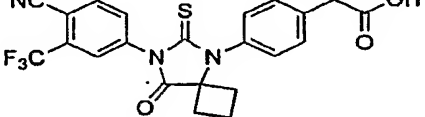
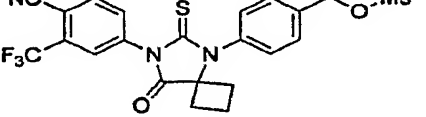
[00133] For several compounds, there was insufficient experimental data to rank them. These untiered compounds are presented in Table 11.

[00134] Based on the data and methods of the invention, and applying judgment based on review
5 of many compounds, including some not shown here, one can make some observations about the untiered compounds. Comparative example NC108 is expected to be in Tier 3 with comparative examples NC78-NC80. NC113 is expected to hydrolyze to NC7 (Tier 1), and should therefore have comparable activity. NC114 is expected to hydrolyze to NC16 (Tier 1), and should therefore have comparable activity. NC115 is
10 expected to hydrolyze to NC3 (Tier 1), and NC120 and NC121 are expected to hydrolyze to NC50 (Tier 2), and they should therefore have comparable activity.

TABLE 11

| UNTIERED COMPOUNDS | |
|--|--|
| <p><i>NC108</i></p>  <p>(comparative)</p> | <p><i>NC109</i></p>  |
| <p><i>NC110</i></p>  | <p><i>NC110</i></p>  |
| <p><i>NC111</i></p>  | <p><i>NC112</i></p>  |
| <p><i>NC113</i></p>  | <p><i>NC114</i></p>  |
| <p><i>NC115</i></p>  | <p><i>NC116</i></p>  |
| <p><i>NC117</i></p>  | <p><i>NC118</i></p>  |

UNTIERED COMPOUNDS

| | |
|---|--|
| <i>NC119</i>  | <i>NC120</i>  |
| <i>NC121</i>  | <i>NC122</i>  |
| <i>NC123</i>  | <i>NC124</i>  |
| <i>NC125</i>  | <i>NC126</i>  |

[00135] In short, novel compounds which show evidence of being far superior to bicalutamide in treating prostate cancer were identified and produced.

5 Sensitivity of Anti-Cancer Activity of Compounds to Structural Differences

[00136] The inventors have determined that what might appear to be a small change in the structure of hydantoin compounds may result in a large change in that compound's performance in treating prostate cancer. For example, NC77 and NC53 differ only by a single fluorine substituent on an aryl ring, and NC53 is in Tier 1, while NC77 is in Tier 2, both being better than bicalutamide for the treatment of prostate cancer, but NC53 being superior. However, NC98, which differs from NC77 only in having an
10 additional carbon atom between the methylcarbamoyl group and the aryl ring, is no better than bicalutamide for the treatment of prostate cancer and is ranked in Tier 4. The effect of NC77, NC53, and NC98 on luciferase activity can be seen in Figure 25. At a given concentration of compound, the luciferase activity upon exposure to NC77 and NC53 is less than the luciferase activity upon exposure to NC98.

15 [00137] NC4 differs from NC3 only in that an amino group is substituted for a hydroxyl group. However, whereas NC3 is in Tier 1, much better than bicalutamide for the treatment of prostate cancer, NC4 is in Tier 4, no better than bicalutamide. The effect of NC3 and NC4 on luciferase activity in the 1AR cell line was studied by administering various compounds at concentrations ranging from 1.25 to 10 μ mol and observing PSA levels. For a given dose, the luciferase activity upon exposure to NC3 is less than the
20 luciferase activity upon exposure to NC4. The effect of NC3 and NC4 on luciferase activity in the 4AR cell line was studied by administering various compounds at concentrations ranging from 1.25 to 10 μ mol and observing luciferase activity. For a given dose, the luciferase activity upon exposure to NC3 is less than the luciferase activity upon exposure to NC4. The effect of NC3 and NC4 on PSA levels in the LN/AR cell line was studied by administering various compounds at concentrations ranging from 1.25 to 10 μ mol and
25 observing luciferase activity. For a given dose, the PSA level upon exposure to NC3 is less than the PSA level upon exposure to NC4.

[00138] NC47 and NC48 differ from each other only by a methyl substituent on the end of a carbamoyl group and both compounds are ranked in Tier 1, although NC48 has been found to be particularly advantageous. NC46 is the same as NC47, with the exception of a methoxy group being substituted for an

amino group. However, NC46 is ranked in Tier 3. NC42 is similar to NC46, but has one less carbon in the chain linking the ester group to the aryl ring; NC42 is ranked in Tier 3. The effect of NC47, NC48, NC42, and NC46 on PSA levels in the LN/AR cell line was studied by administering various compounds at concentrations ranging from 125 nmol to 1000 nmol and observing PSA levels. For a given concentration, the PSA level upon exposure to NC47 and NC48 is less than the PSA level upon exposure to NC42 and NC46.

[00139] NC68 and NC103 differ from each other in that the former has a methylcarbamoyl group attached to an aryl ring and a dimethyl substituent attached to the thiohydantoin group, whereas the latter has a methylamino group attached to the right hand aryl ring and a cyclobutyl substituent attached to the thiohydantoin group. Whereas NC68 is in Tier 1, much better than bicalutamide for the treatment of prostate cancer, NC103 is in Tier 5, inactive or nearly inactive in the treatment of prostate cancer. The effect of NC68 and NC103 on luciferase activity in the LN/AR cell line was studied by administering various compounds at concentrations ranging from 125 nmol to 1000 nmol and observing luciferase activity. For a given concentration, the luciferase activity upon exposure to NC68 is less than the luciferase activity upon exposure to NC103.

[00140] NC16 and NC18 differ from each other in the substitution of a thio for an oxo group and a dimethyl substituent for a cyclobutyl substituent. Whereas NC16 is in Tier 1, NC18 is in Tier 4.

Pharmaceutical Compositions and Administration

[00141] The compounds of the invention are useful as pharmaceutical compositions prepared with a therapeutically effective amount of a compound of the invention, as defined herein, and a pharmaceutically acceptable carrier or diluent.

[00142] The diarylhydantoin compounds of the invention can be formulated as pharmaceutical compositions and administered to a subject in need of treatment, for example a mammal, such as a human patient, in a variety of forms adapted to the chosen route of administration, for example, orally, nasally, intraperitoneally, or parenterally, by intravenous, intramuscular, topical or subcutaneous routes, or by injection into tissue.

[00143] Thus, diarylhydantoin compounds of the invention may be systemically administered, e.g., orally, in combination with a pharmaceutically acceptable vehicle such as an inert diluent or an assimilable edible carrier, or by inhalation or insufflation. They may be enclosed in hard or soft shell gelatin capsules,

may be compressed into tablets, or may be incorporated directly with the food of the patient's diet. For oral therapeutic administration, the diarylhydantoin compounds may be combined with one or more excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. The diarylhydantoin compounds may be combined with a fine inert powdered carrier and inhaled by the subject or insufflated. Such compositions and preparations should contain at least 0.1% diarylhydantoin compounds. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2% to about 60% of the weight of a given unit dosage form. The amount of diarylhydantoin compounds in such therapeutically useful compositions is such that an effective dosage level will be obtained.

[00144] The tablets, troches, pills, capsules, and the like may also contain the following: binders such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, fructose, lactose or aspartame or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring may be added. When the unit dosage form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials may be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, wax, shellac or sugar and the like. A syrup or elixir may contain the active compound, sucrose or fructose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any unit dosage form should be pharmaceutically acceptable and substantially non-toxic in the amounts employed. In addition, the diarylhydantoin compounds may be incorporated into sustained-release preparations and devices. For example, the diarylhydantoin compounds may be incorporated into time release capsules, time release tablets, and time release pills.

[00145] The diarylhydantoin compounds may also be administered intravenously or intraperitoneally by infusion or injection. Solutions of the diarylhydantoin compounds can be prepared in water, optionally mixed with a nontoxic surfactant. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, triacetin, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations can contain a preservative to prevent the growth of microorganisms.

[00146] The pharmaceutical dosage forms suitable for injection or infusion can include sterile aqueous solutions or dispersions or sterile powders comprising the diarylhydantoin compounds which are

adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions, optionally encapsulated in liposomes. In all cases, the ultimate dosage form should be sterile, fluid and stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol (for example, glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the formation of liposomes, by the maintenance of the required particle size in the case of dispersions or by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, buffers or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

[00147] Sterile injectable solutions are prepared by incorporating the diarylhydantoin compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze drying techniques, which yield a powder of the active ingredient plus any additional desired ingredient present in the previously sterile-filtered solutions.

[00148] For topical administration, the diarylhydantoin compounds may be applied in pure form. However, it will generally be desirable to administer them to the skin as compositions or formulations, in combination with a dermatologically acceptable carrier, which may be a solid or a liquid.

[00149] Useful solid carriers include finely divided solids such as talc, clay, microcrystalline cellulose, silica, alumina and the like. Other solid carriers include nontoxic polymeric nanoparticles or microparticles. Useful liquid carriers include water, alcohols or glycols or water/alcohol/glycol blends, in which the diarylhydantoin compounds can be dissolved or dispersed at effective levels, optionally with the aid of non-toxic surfactants. Adjuvants such as fragrances and additional antimicrobial agents can be added to optimize the properties for a given use. The resultant liquid compositions can be applied from absorbent pads, used to impregnate bandages and other dressings, or sprayed onto the affected area using pump-type or aerosol sprayers.

[00150] Thickeners such as synthetic polymers, fatty acids, fatty acid salts and esters, fatty

alcohols, modified celluloses or modified mineral materials can also be employed with liquid carriers to form spreadable pastes, gels, ointments, soaps, and the like, for application directly to the skin of the user.

[00151] Examples of useful dermatological compositions which can be used to deliver the diarylhydantoin compounds to the skin are known to the art; for example, see Jacquet et al. (U.S. Pat. No. 4,608,392), Geria (U.S. Pat. No. 4,992,478), Smith et al. (U.S. Pat. No. 4,559,157) and Wortzman (U.S. Pat. No. 4,820,508), all of which are hereby incorporated by reference.

[00152] Useful dosages of the compounds of formula I can be determined by comparing their in vitro activity, and in vivo activity in animal models. Methods for the extrapolation of effective dosages in mice, and other animals, to humans are known to the art; for example, see U.S. Pat. No. 4,938,949, which is hereby incorporated by reference.

[00153] For example, the concentration of the diarylhydantoin compounds in a liquid composition, such as a lotion, can be from about 0.1-25% by weight, or from about 0.5-10% by weight. The concentration in a semi-solid or solid composition such as a gel or a powder can be about 0.1-5% by weight, or about 0.5-2.5% by weight.

[00154] The amount of the diarylhydantoin compounds required for use in treatment will vary not only with the particular salt selected but also with the route of administration, the nature of the condition being treated and the age and condition of the patient and will be ultimately at the discretion of the attendant physician or clinician.

[00155] Effective dosages and routes of administration of agents of the invention are conventional. The exact amount (effective dose) of the agent will vary from subject to subject, depending on, for example, the species, age, weight and general or clinical condition of the subject, the severity or mechanism of any disorder being treated, the particular agent or vehicle used, the method and scheduling of administration, and the like. A therapeutically effective dose can be determined empirically, by conventional procedures known to those of skill in the art. See, e.g., *The Pharmacological Basis of Therapeutics*, Goodman and Gilman, eds., Macmillan Publishing Co., New York. For example, an effective dose can be estimated initially either in cell culture assays or in suitable animal models. The animal model may also be used to determine the appropriate concentration ranges and routes of administration. Such information can then be used to determine useful doses and routes for administration in humans. A therapeutic dose can also be selected by analogy to dosages for comparable therapeutic agents.

[00156] The particular mode of administration and the dosage regimen will be selected by the attending clinician, taking into account the particulars of the case (*e.g.*, the subject, the disease, the disease state involved, and whether the treatment is prophylactic). Treatment may involve daily or multi-daily doses of compound(s) over a period of a few days to months, or even years.

5 [00157] In general, however, a suitable dose will be in the range of from about 0.001 to about 100 mg/kg, *e.g.*, from about 0.01 to about 100 mg/kg of body weight per day, such as above about 0.1 mg per kilogram, or in a range of from about 1 to about 10 mg per kilogram body weight of the recipient per day. For example, a suitable dose may be about 1 mg/kg, 10 mg/kg, or 50 mg/kg of body weight per day.

[00158] The diarylhydantoin compounds are conveniently administered in unit dosage form; for
10 example, containing 0.05 to 10000 mg, 0.5 to 10000 mg, 5 to 1000 mg, or about 100 mg of active ingredient per unit dosage form.

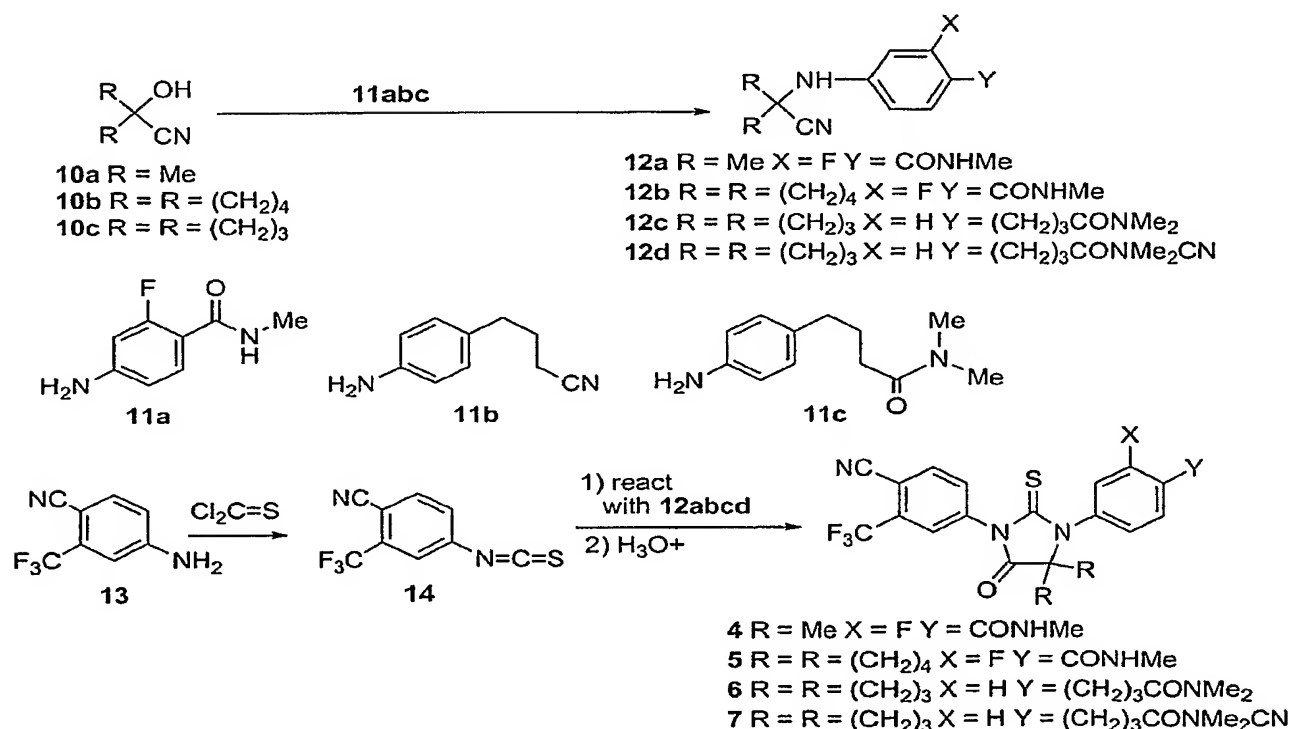
[00159] The diarylhydantoin compounds can be administered to achieve peak plasma concentrations of, for example, from about 0.5 to about 75 μ M, about 1 to 50 μ M, about 2 to about 30 μ M, or about 5 to about 25 μ M. Exemplary desirable plasma concentrations include at least or no more than 0.25,
15 0.5, 1, 5, 10, 25, 50, 75, 100 or 200 μ M. For example, plasma levels may be from about 1 to 100 micromolar or from about 10 to about 25 micromolar. This may be achieved, for example, by the intravenous injection of a 0.05 to 5% solution of the diarylhydantoin compounds, optionally in saline, or orally administered as a bolus containing about 1-100 mg of the diarylhydantoin compounds. Desirable blood levels may be
20 maintained by continuous infusion to provide about 0.00005 - 5 mg per kg body weight per hour, for example at least or no more than 0.00005, 0.0005, 0.005, 0.05, 0.5, or 5 mg/kg/hr. Alternatively, such levels can be obtained by intermittent infusions containing about 0.0002 - 20 mg per kg body weight, for example, at least or no more than 0.0002, 0.002, 0.02, 0.2, 2, 20, or 50 mg of the diarylhydantoin compounds per kg of body weight.

[00160] The diarylhydantoin compounds may conveniently be presented in a single dose or as
25 divided doses administered at appropriate intervals, for example, as two, three, four or more sub-doses per day. The sub-dose itself may be further divided, *e.g.*, into a number of discrete loosely spaced administrations; such as multiple inhalations from an insufflator.

[00161] A number of the above-identified compounds exhibit little or no agonistic activities with respect to hormone refractory prostate cancer cells. Because these compounds are strong AR inhibitors, they
30 can be used not only in treating prostate cancer, but also in treating other AR related diseases or conditions

such as benign prostate hyperplasia, hair loss, and acne. Because AR belongs to the family of nuclear receptors, these compounds may serve as scaffolds for drug synthesis targeting other nuclear receptors, such as estrogen receptor and peroxisome proliferator-activated receptor. Therefore, they may be further developed for other diseases such as breast cancer, ovarian cancer, diabetes, cardiac diseases, and metabolism related diseases, in which nuclear receptors play a role.

[00162] A sequence for the chemical synthesis of several compounds according to the invention is shown below. The cyanohydrins **10abc** are converted into the four different cyanoamines **12abcd** by reaction with the three different anilines **11abc** (**10a** and **11a** give **12a**, **10b** and **11a** give **12b**, **10c** and **11b** give **12c**, and **10c** and **11c** give **12d**). In a separate process the aniline **13** is converted in one step into the isothiocyanate **14**. Addition of **12abcd** to **14** followed by treatment with mild acid produces the desired thiohydantoin **4** (NC54), **5** (NC55), **6** (NC56), and **7** in good yield.



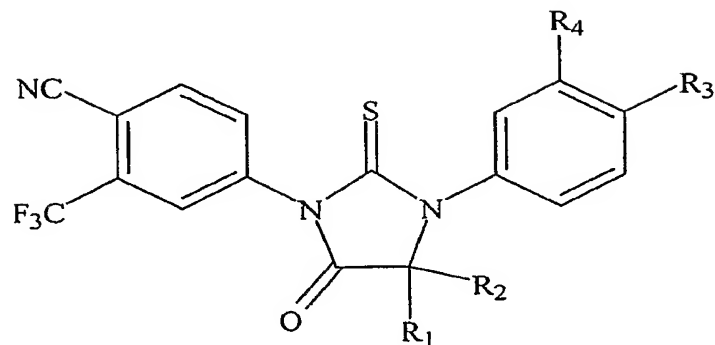
[00163]

[00164] The embodiments illustrated and discussed in this specification are intended only to teach those skilled in the art the best way known to the inventors to make and use the invention. Nothing in this
5 specification should be considered as limiting the scope of the present invention. All examples presented are representative and non-limiting. The above-described embodiments of the invention may be modified or varied, without departing from the invention, as appreciated by those skilled in the art in light of the above teachings. It is therefore to be understood that, within the scope of the claims and their equivalents, the invention may be practiced otherwise than as specifically described.

10

WE CLAIM:

1. A compound having the formula



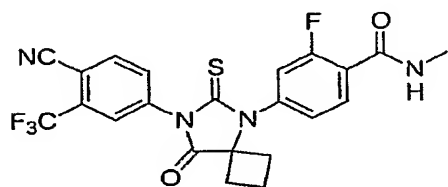
wherein R_1 and R_2 are independently methyl or, together with the carbon to which they are linked, a cycloalkyl group of 4 to 5 carbon atoms,

wherein R_3 is selected from the group consisting of carbamoyl, alkylcarbamoyl, carbamoylalkyl, alkylcarbamoylalkyl, cyano, and cyanoalkyl, and

wherein R_4 is hydrogen or fluorine.

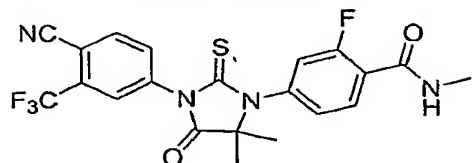
2. A pharmaceutical composition comprising a therapeutically effective amount of a compound according to claim 1 or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier or diluent.

3. The compound of claim 1, having the formula



[NC53]

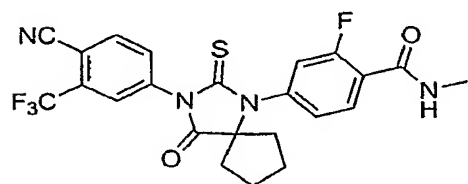
4. The compound of claim 1, having the formula



[NC54]

5. A pharmaceutical composition comprising a therapeutically effective amount of a compound according to claim 4 or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier or diluent.

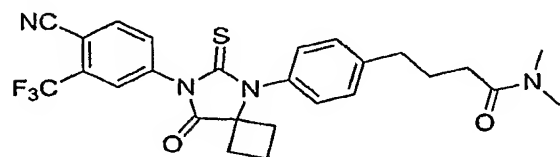
6. The compound of claim 1, having the formula



[NC55]

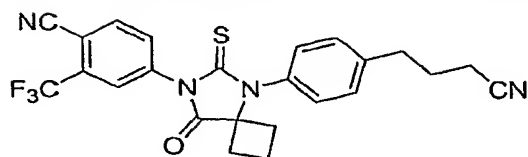
7. A pharmaceutical composition comprising a therapeutically effective amount of a compound according to claim 6 or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier or diluent.

8. The compound of claim 1, having the formula



[NC56]

9. The compound of claim 1, having the formula



[NC57]

10. A method for treating a hyperproliferative disorder comprising administering a pharmaceutical composition of claim 2 to a subject in need of such treatment, thereby treating the hyperproliferative disorder.

11. The method of claim 10, wherein the composition has a form selected from the group consisting of a solution, dispersion, suspension, powder, capsule, tablet, pill, time release capsule, time release tablet, and time release pill.

12. The method of claim 10, wherein the compound is administered by intravenous injection, by injection into tissue, intraperitoneally, orally, or nasally.

13. The method of claim 10, wherein the composition is administered at a dosage of the compound in the range of from about 0.001 mg per kg body weight per day to about 100 mg per kg body weight per day.

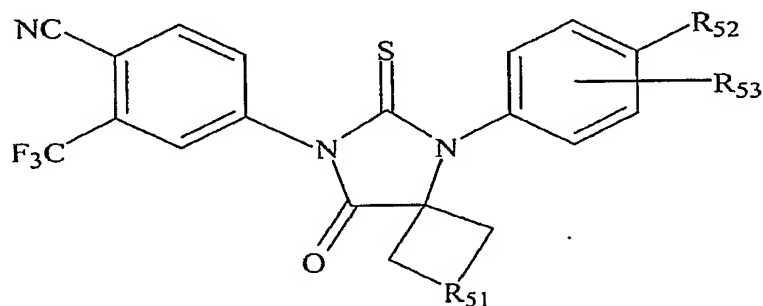
14. The method of claim 10, wherein the composition is administered at a dosage of the compound in the range of from about 0.01 mg per kg body weight per day to about 100 mg per kg body weight per day.

15. The method of claim 10, wherein the composition is administered at a dosage of the compound in the range of from about 0.1 mg per kg body weight per day to about 10 mg per kg body weight per day.

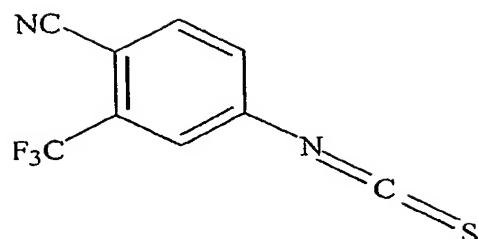
16. The method of claim 10, wherein the composition is administered at a dosage of the compound of about 1 mg per kg body weight per day.

17. A method for treating prostate cancer comprising administering a composition according to paragraph 2 to a subject in need of such treatment, thereby treating the prostate cancer.

18. The method of claim 10, wherein the pharmaceutical composition interferes with the transcription of prostate specific antigen mRNA.
19. The method of claim 10, wherein the pharmaceutical composition prevents nuclear translocation of an androgen receptor protein.
20. The method of claim 10, wherein the pharmaceutical composition destabilizes an androgen receptor protein.
21. The method of claim 10, wherein the composition is administered orally.
22. The method of claim 10, wherein the composition has a form selected from the group consisting of a capsule, tablet, and pill.
23. The method of claim 10, wherein the compound is selected from the group consisting of NC54, NC55, NC56, NC57, a pharmaceutically acceptable salt of any of these, and combinations thereof.
24. The method of claim 10, wherein the compound is NC53 or a pharmaceutically acceptable salt.
25. A method of synthesizing a diaryl compound of formula:

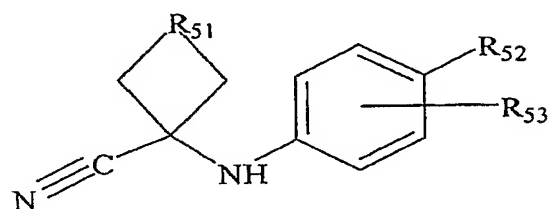


comprising mixing Compound I



Compound I

with Compound II



Compound II

in a first polar solvent to form a mixture,

heating the mixture,

adding a second polar solvent, the same as or different from the first polar solvent, and an aqueous acid to the mixture,

refluxing the mixture,

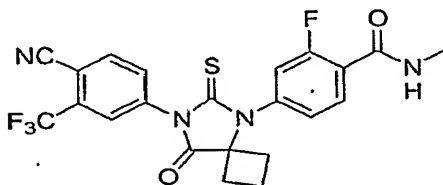
cooling the mixture and combining with water, and

separating the diaryl compound from the mixture,

wherein R51 comprises an alkyl chain of from 1 to 4 carbon atoms, R52 is selected from the group consisting of cyano, hydroxy, methylcarbamoyl, methylcarbamoyl-substituted alkyl, methylsulfonocarbamoyl-substituted alkyl, methylaminomethyl, dimethylaminomethyl, methylsulfonyloxymethyl, methoxycarbonyl, 3-cyano-4-trifluoromethylphenylcarbamoyl, carbamoyl-substituted alkyl, carboxymethyl, methoxycarbonylmethyl, methanesulfonyl, 4-cyano-3-trifluoromethylphenylcarbamoyl-substituted alkyl, carboxy-substituted alkyl, 4-methanesulfonyl-1-piperazinyl, piperazinyl, hydroxyethylcarbamoyl-substituted alkyl, and hydroxyethoxycarbonyl-substituted alkyl, and R53 is selected from the group consisting of F and H.

26. The method of claim 25, wherein R51 comprises an alkyl chain of from 1 to 2 carbon atoms, R52 is selected from the group consisting of carbamoyl and methylcarbamoyl, and R53 is F.

27. A method of synthesizing a compound of formula:



[NC53]

comprising

mixing 4-isothiocyanto-2-trifluoromethylbenzonitrile and *N*-methyl-4-(1-cyanocyclobutylamino)-2-fluorobenzamide in dimethylformamide to form a first mixture,
 heating the first mixture to form a second mixture,
 adding alcohol and acid to the second mixture to form a third mixture,
 refluxing the third mixture to form a fourth mixture,
 cooling the fourth mixture,
 combining the fourth mixture with water and extracting an organic layer;
 isolating the compound from the organic layer.

28. A method of synthesizing the compound of claim 4 [NC54], comprising
 mixing *N*-methyl-2-fluoro-4-(1,1-dimethyl-cyanomethyl)-aminobenzamide and 4-isothiocyanto-2-trifluoromethylbenzonitrile in DMF and heating to form a first mixture;
 adding an alcohol and an acid to the first mixture to form a second mixture;
 refluxing the second mixture;
 cooling the second mixture,
 combining the second mixture with water and extracting an organic layer;
 isolating the compound from the organic layer.

29. A method of synthesizing the compound of claim 6 [NC55], comprising
 mixing *N*-methyl-2-fluoro-4-(1-cyanocyclopentyl)aminobenzamide, 4-isothiocyanto-2-

trifluoromethyl benzonitrile, and DMF and heating under reflux to form a first mixture;
adding an alcohol and an acid to the first mixture to form a second mixture;
refluxing the second mixture;
cooling the second mixture;
combining the second mixture with water and extracting an organic layer;
isolating the compound from the organic layer.

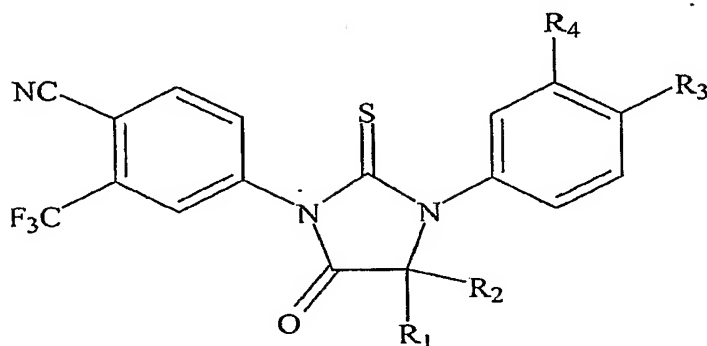
30. A method of synthesizing the compound of claim 8 [NC56], comprising
mixing *N,N*-dimethyl 4-[4-(1-cyanocyclobutylamino)phenyl]butanamide, 4-isothiocyanato-2-trifluoromethyl benzonitrile, and DMF and heating under reflux to form a first mixture;
adding an alcohol and an acid to the first mixture to form a second mixture;
refluxing the second mixture;
cooling the second mixture;
combining the second mixture with water and extracting an organic layer;
isolating the compound from the organic layer.

31. A method of synthesizing the compound of claim 9 [NC57], comprising
mixing DMSO, dichloromethane, and oxalyl chloride to form a first mixture,
adding 4-(4-(7-(4-cyano-3-(trifluoromethyl)phenyl)-8-oxo-6-thioxo-5,7-diazaspiro[3.4]octan-5-yl)phenyl)butanamide to the first mixture to form a second mixture;
adding triethylamine to the second mixture to form a third mixture;
warming the third mixture and quenching with aqueous NH_4Cl to form a fourth mixture;
extracting an organic layer from the fourth mixture;
isolating the compound from the organic layer.

32. A method comprising:
providing at least one diarylthiohydantoin compound;
measuring inhibition of androgen receptor activity for the compound and determining if the inhibition is above a first predetermined level,
measuring stimulation of androgen receptor activity in hormone refractory cancer cells for the compound and determining if the stimulation is below a second predetermined level,
selecting the compound if the inhibition is above the first predetermined level and the stimulation is below the second predetermined level.

33. The method of claim 32, wherein the predetermined levels are those of bicalutamide.
34. The method of claim 32, wherein the step of measuring inhibition comprises measuring inhibitory concentration (IC50) in an AR response reporter system or a prostate specific antigen secreting system.
35. The method of claim 32, wherein the step of measuring stimulation comprises measuring fold induction by increasing concentrations in an AR response reporter system or a prostate specific antigen secreting system.
36. The method of claim 32, wherein the steps of measuring inhibition and/or stimulation comprise measuring an effect of the compound on tumor growth in an animal.
37. The method of claim 32, wherein the step of measuring inhibition and/or stimulation of androgen receptor activity comprises measuring the binding affinity of an androgen receptor to the compound.
38. The method of claim 32, wherein the step of measuring inhibition and/or stimulation of androgen receptor activity comprises measuring prevention of androgen receptor recruitment to at least one of prostate specific antigen enhancer and prostate specific antigen promoter.
39. The method of claim 32, wherein the step of measuring inhibition and/or stimulation of androgen receptor activity comprises measuring prevention of androgen receptor nuclear translocation.
40. The method of claim 32, wherein the step of measuring inhibition and/or stimulation of androgen receptor activity comprises measuring destabilization of an androgen receptor protein.

41. The method of claim 32, wherein the diarylthiohydantoin compound has the formula



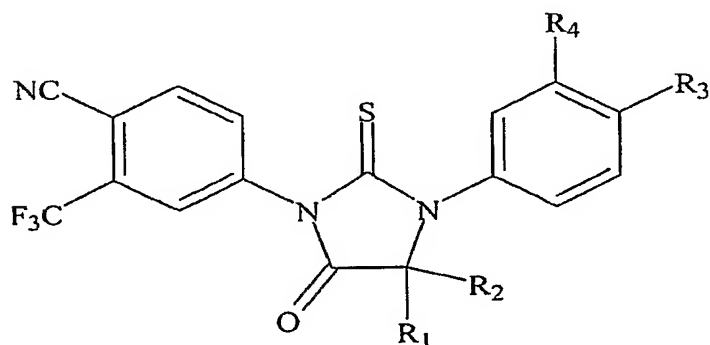
wherein R_1 and R_2 are independently methyl or, together with the carbon to which they are linked, a cycloalkyl group of 4 to 5 carbon atoms,

wherein R_3 is selected from the group consisting of carbamoyl, alkylcarbamoyl, carbamoylalkyl, alkylcarbamoylalkyl, cyano, and cyanoalkyl, and

wherein R_4 is hydrogen or fluorine.

42. A method comprising contacting a mammalian cell capable of expressing prostate specific antigen with a sufficient amount of a diarylthiohydantoin compound to interfere with the transcription of prostate specific antigen mRNA.

43. The method of claim 42, wherein the diarylthiohydantoin compound has the formula



wherein R_1 and R_2 are independently methyl or, together with the carbon to which they are linked, a cycloalkyl group of 4 to 5 carbon atoms,

wherein R_3 is selected from the group consisting of carbamoyl, alkylcarbamoyl, carbamoylalkyl, alkylcarbamoylalkyl, cyano, and cyanoalkyl, and
wherein R_4 is hydrogen or fluorine.

44. The method of claim 42, wherein the diarylthiohydantoin compound is selected from the group consisting of NC53, NC54, NC55, NC56, and NC57.

45. The method of claim 42, wherein the compound prevents formation of a transcription complex on a prostate specific antigen gene.

46. The method of claim 42, wherein the compound prevents an androgen receptor protein from complexing with a prostate specific antigen gene.

47. The method of claim 42, wherein the compound prevents an RNA polymerase II from complexing with a prostate specific antigen gene.

48. A method, comprising contacting a mammalian cell with a sufficient amount of a diarylthiohydantoin compound to prevent nuclear translocation of an androgen receptor protein and/or to destabilize an androgen receptor protein.

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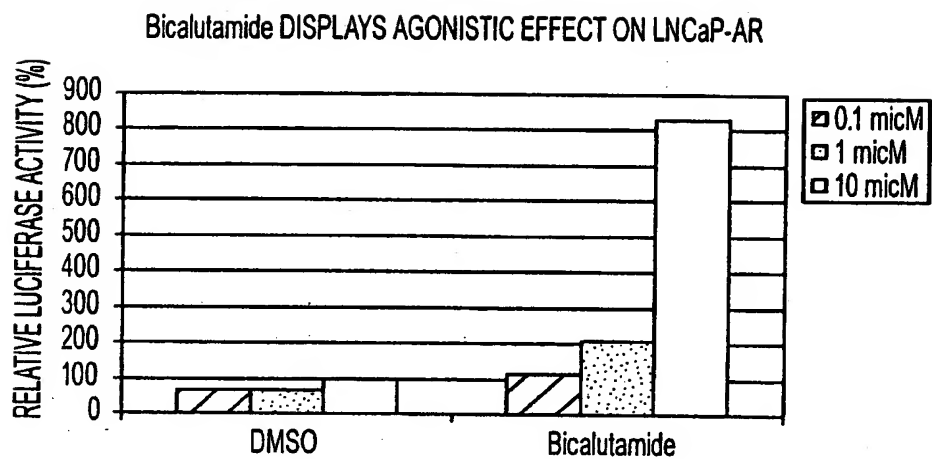


FIG. 1

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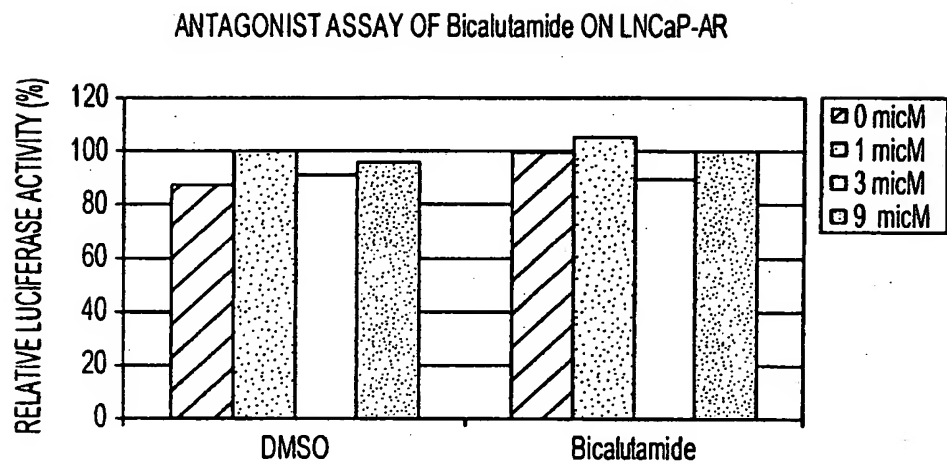


FIG. 2

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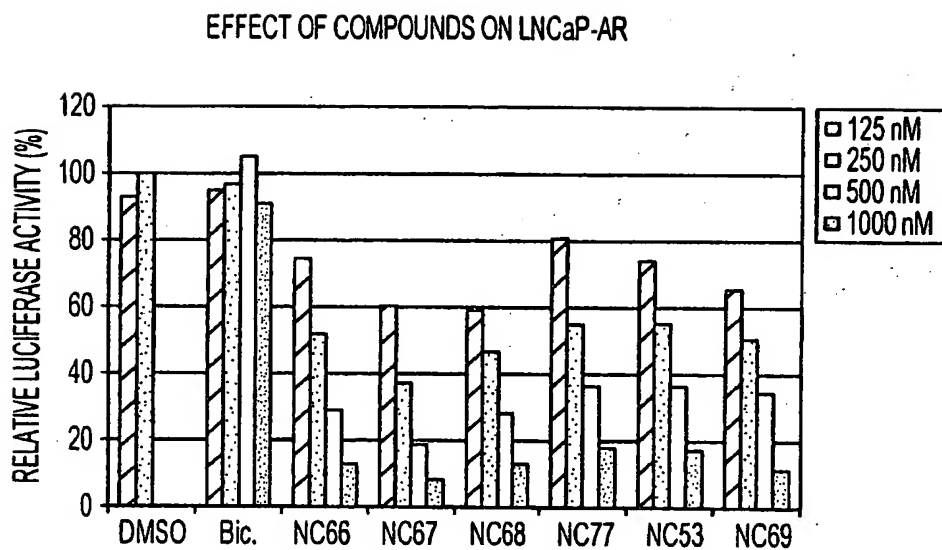


FIG. 3

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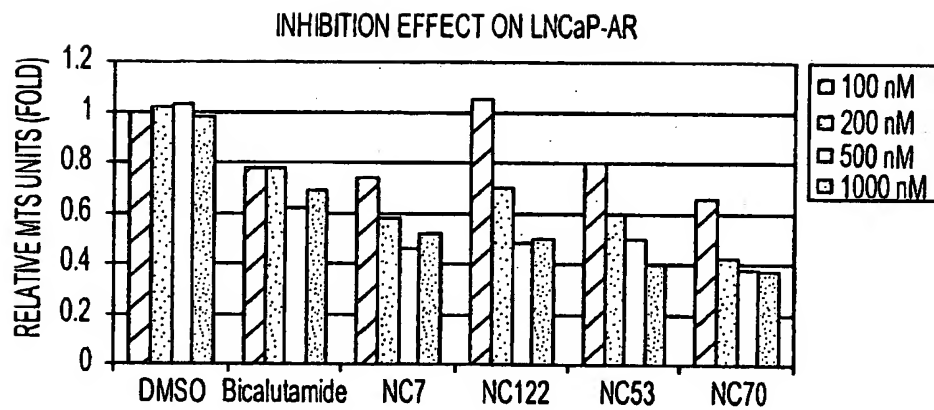


FIG. 4

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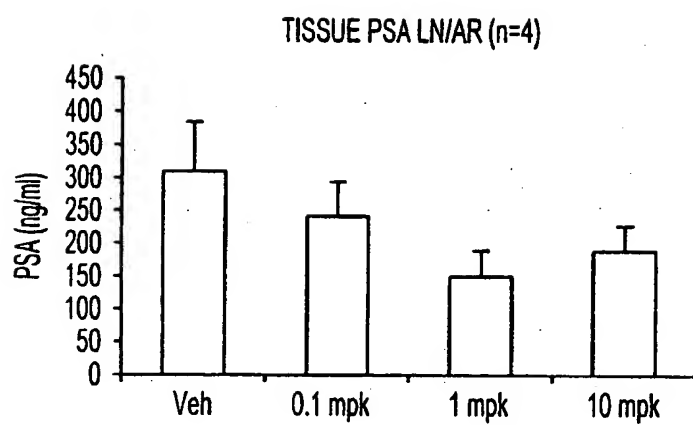


FIG. 5

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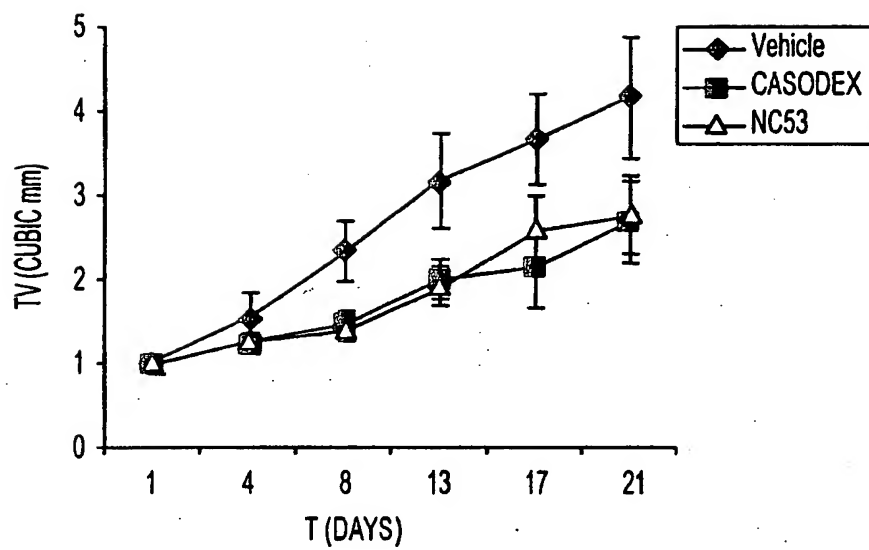


FIG. 6

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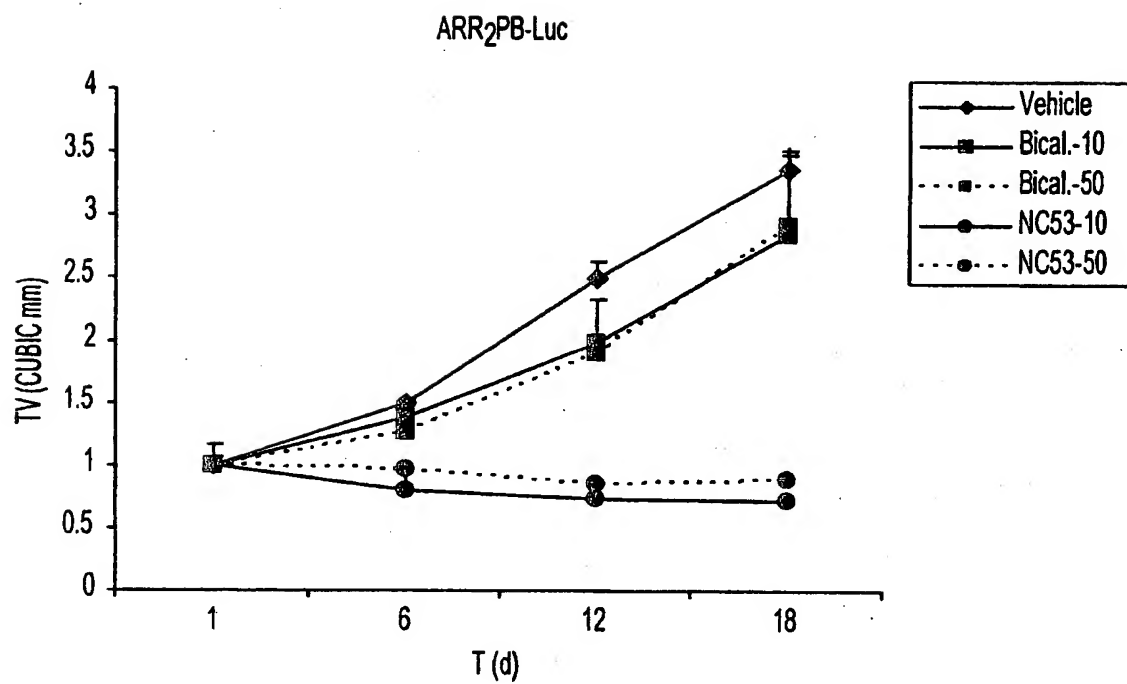


FIG. 7

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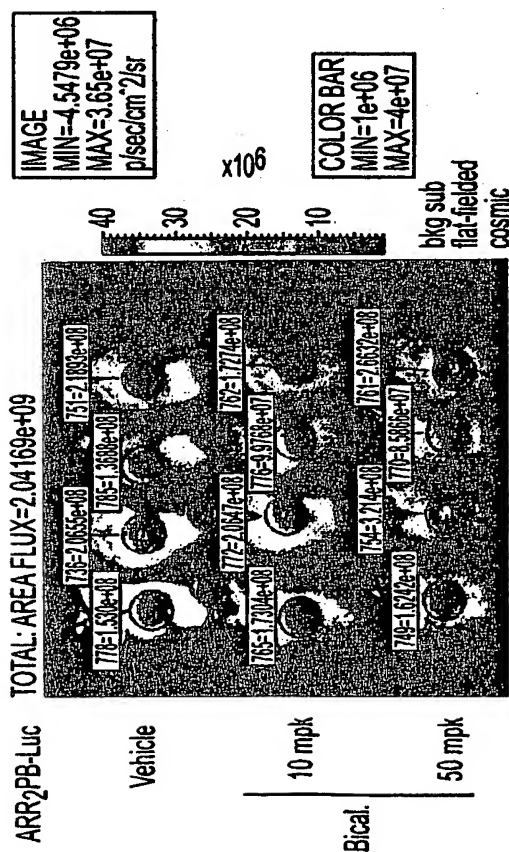


FIG. 8A

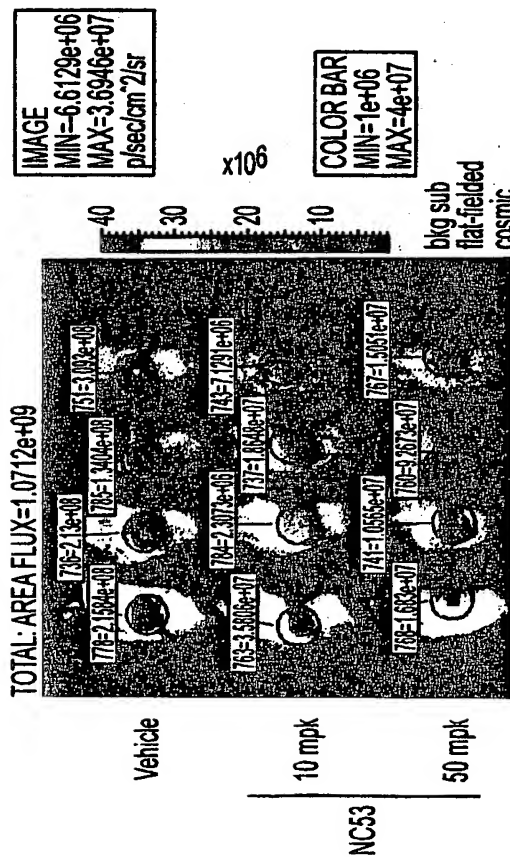


FIG. 8B

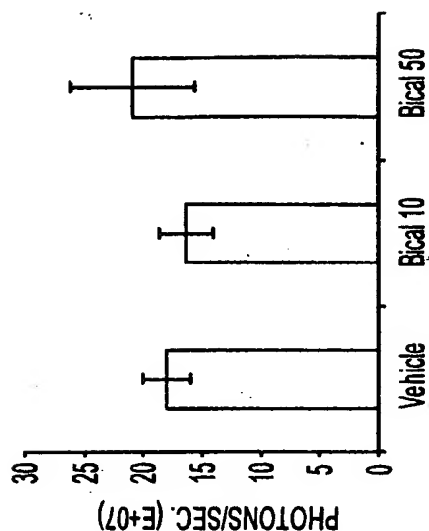


FIG. 8C

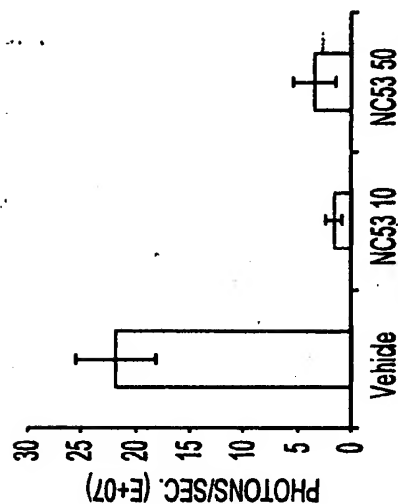


FIG. 8D

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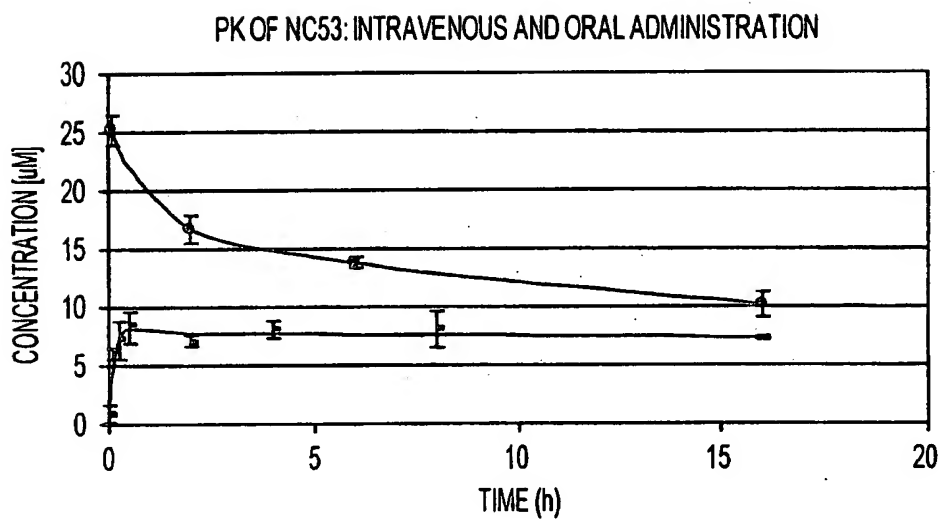


FIG. 9

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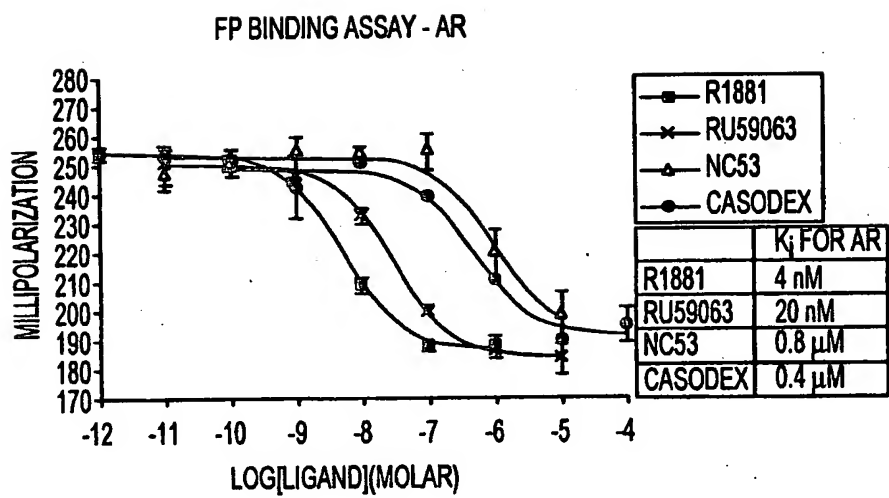


FIG. 10

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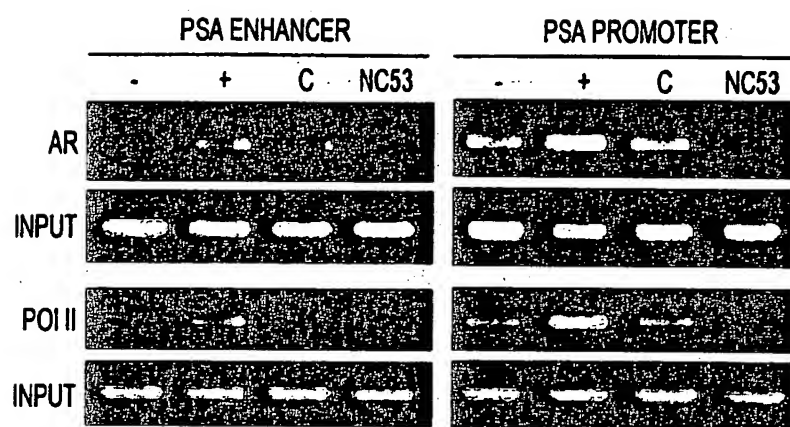


FIG. 11

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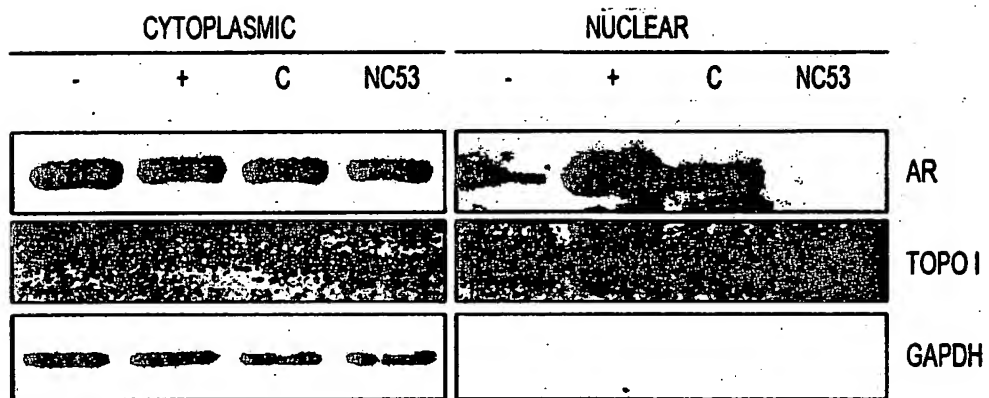


FIG. 12

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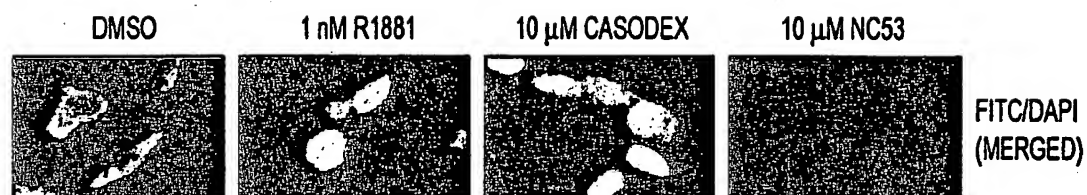


FIG. 13

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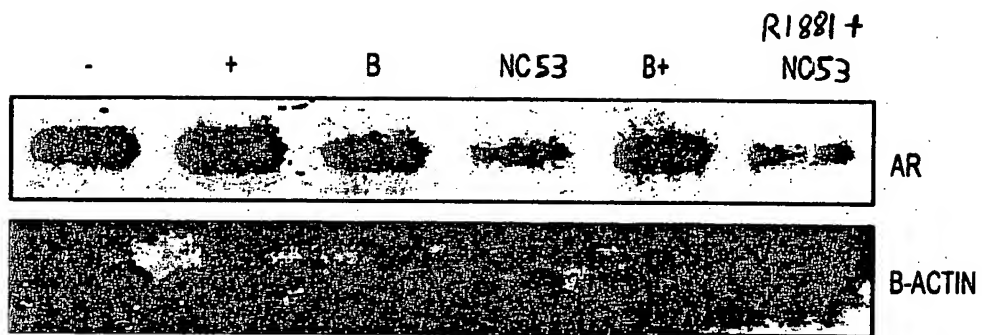


FIG. 14

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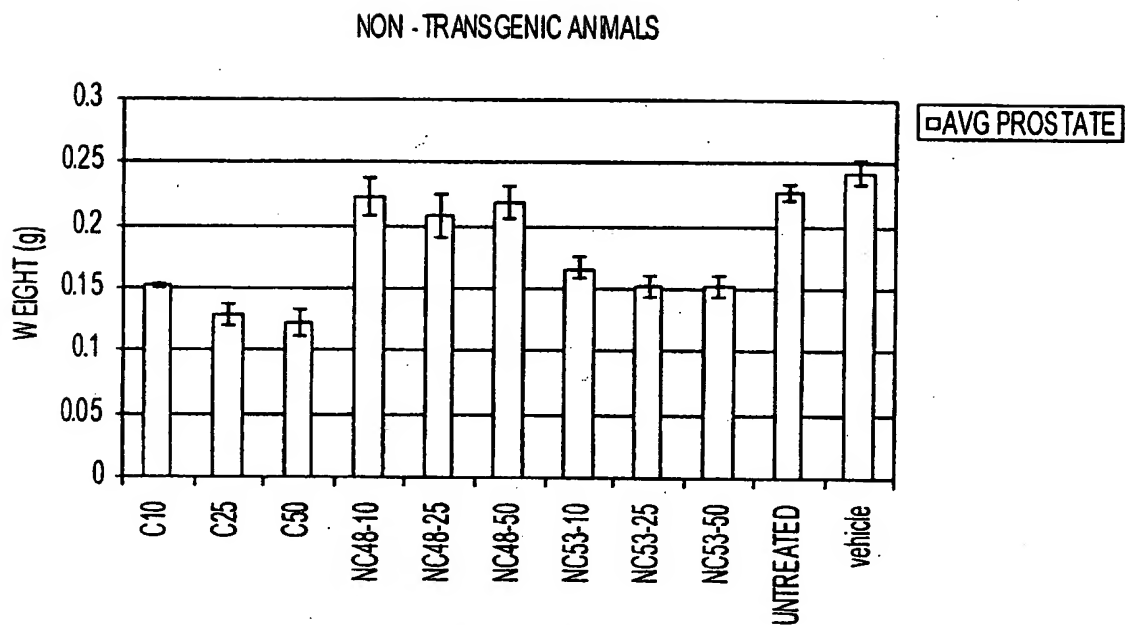


FIG. 15

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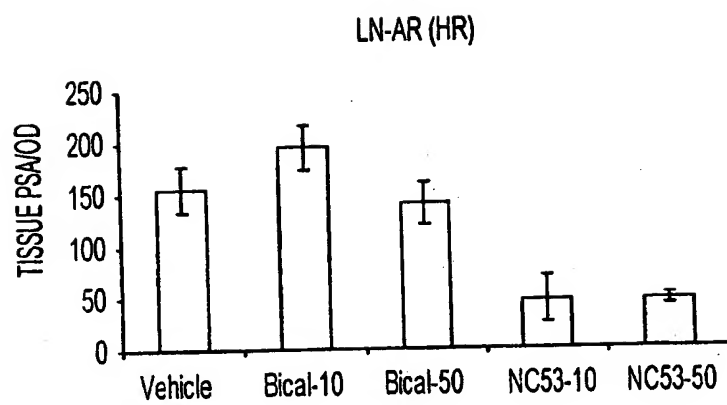


FIG. 16

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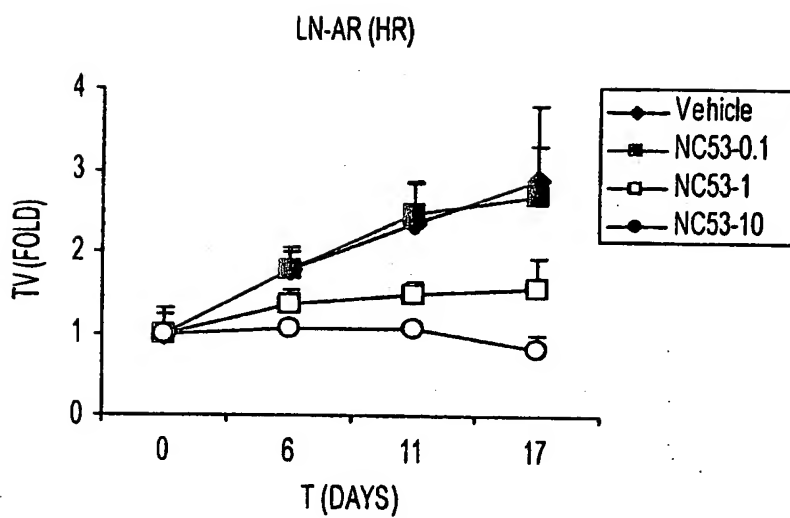


FIG. 17

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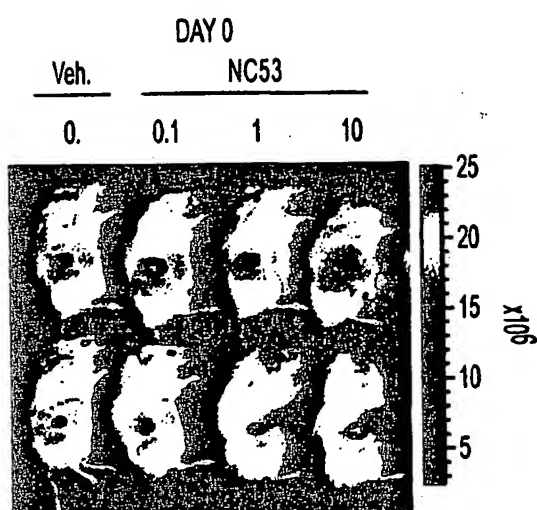


FIG. 18A

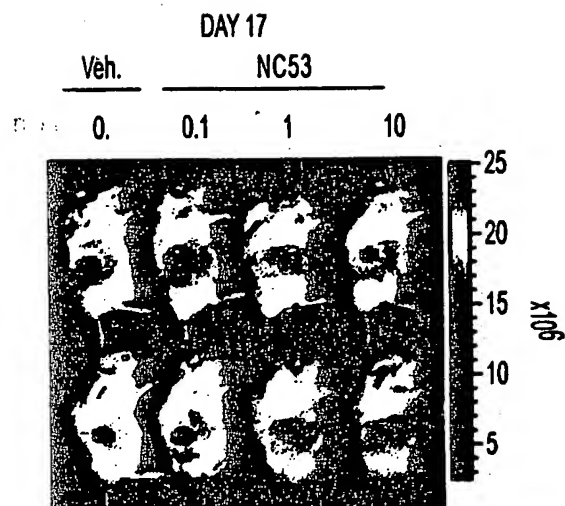


FIG. 18B

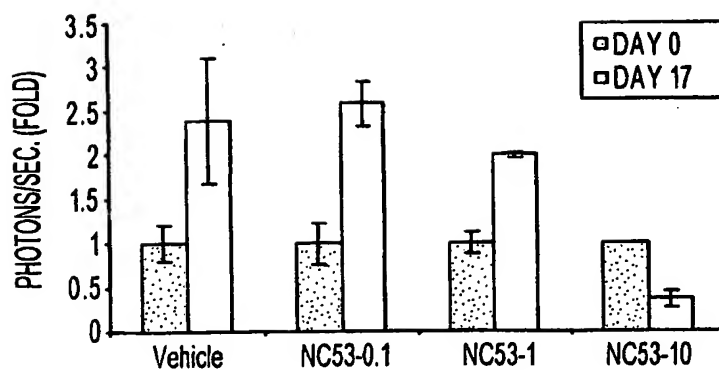


FIG. 18C

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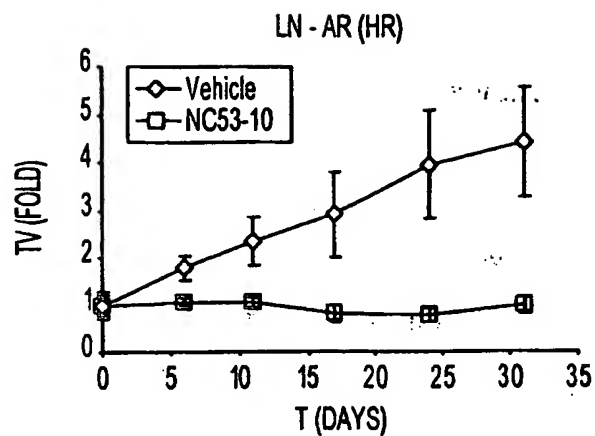


FIG. 19A

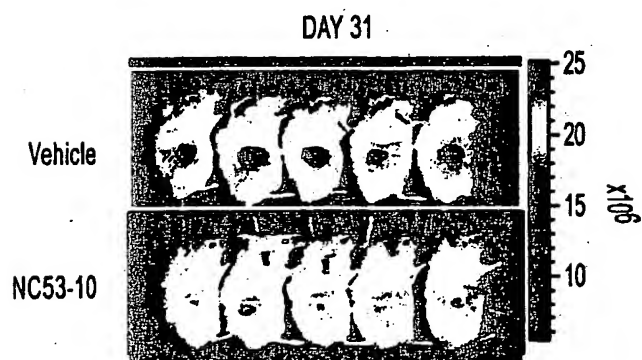


FIG. 19B

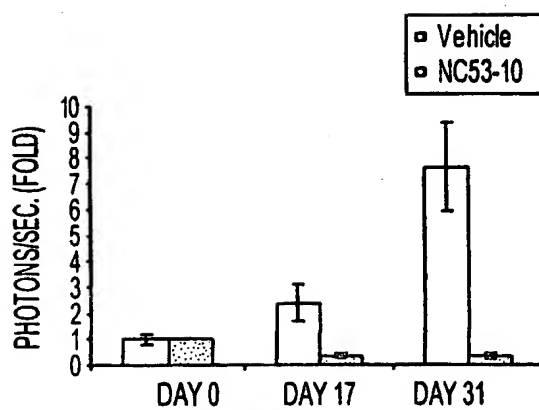


FIG. 19C

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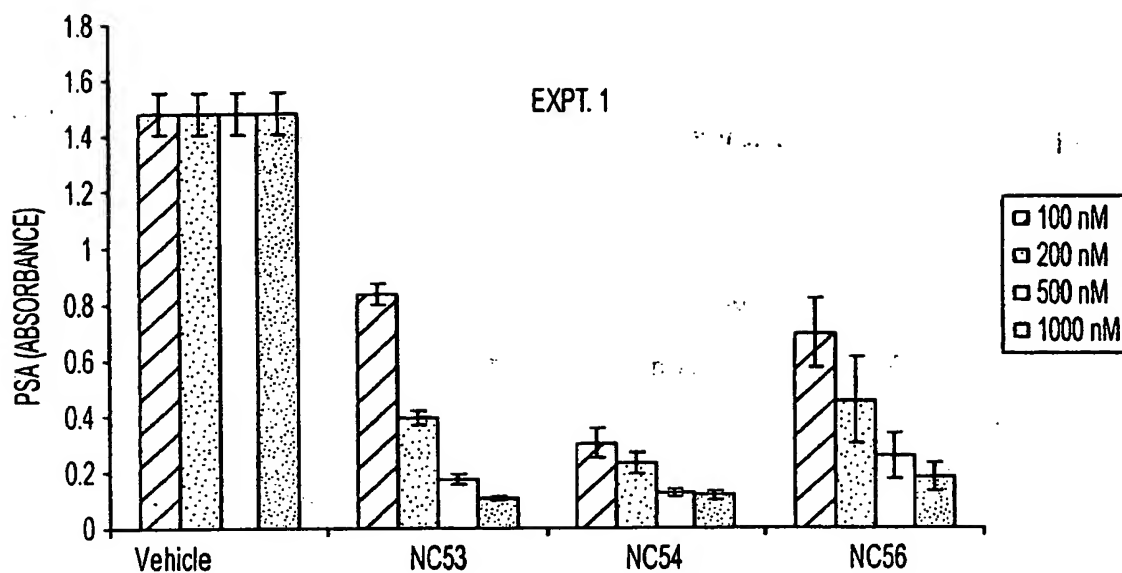


FIG. 20A

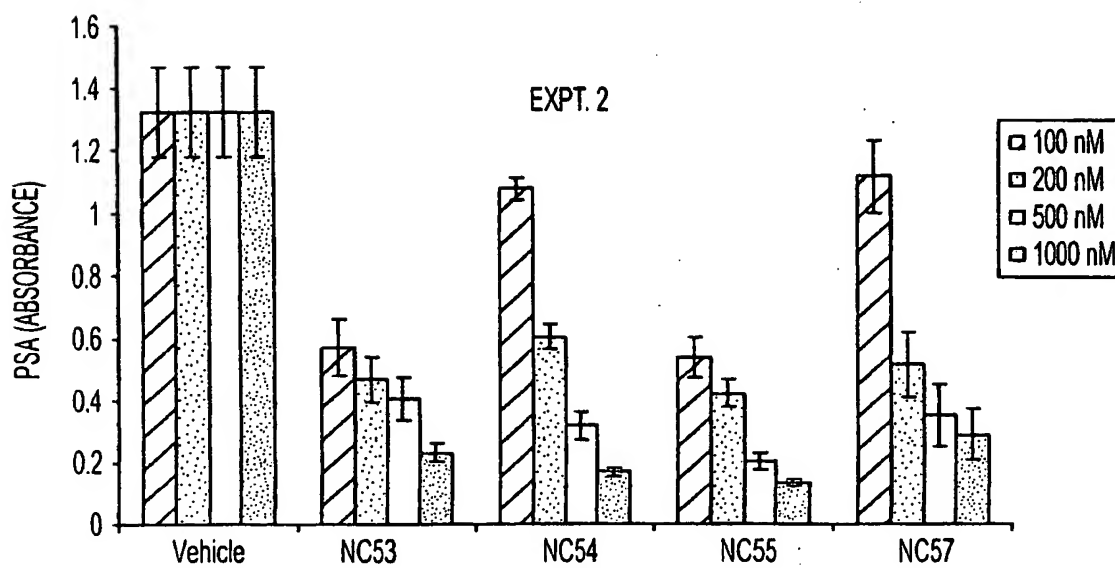


FIG. 20B

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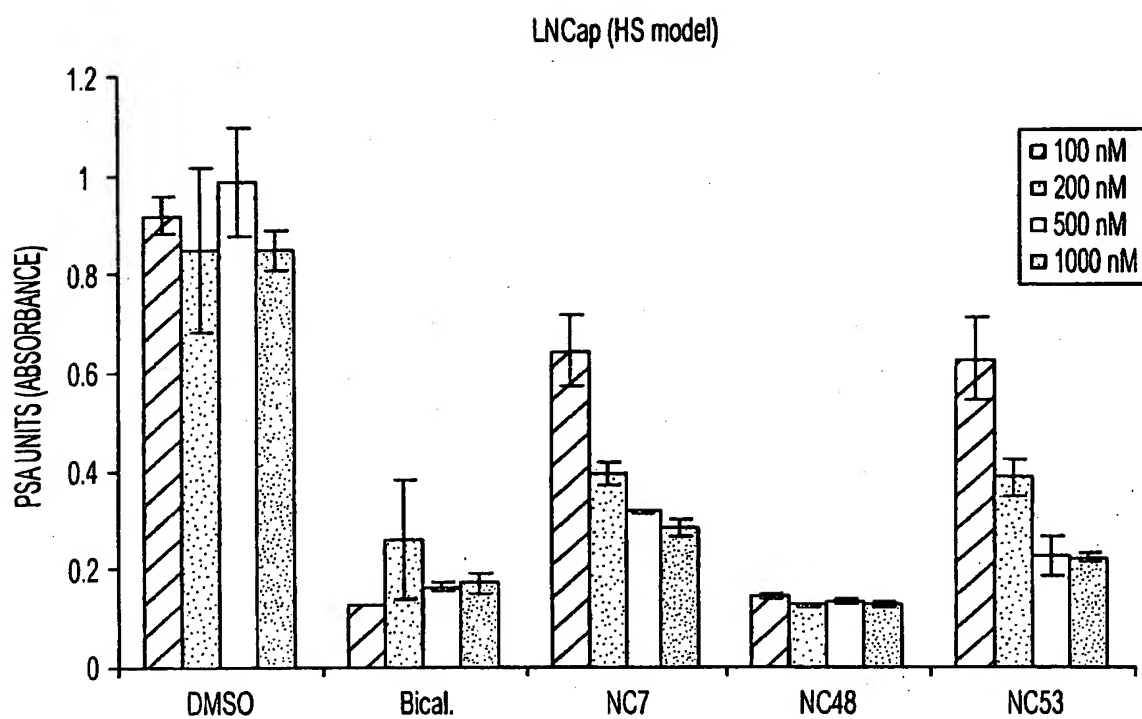


FIG. 21

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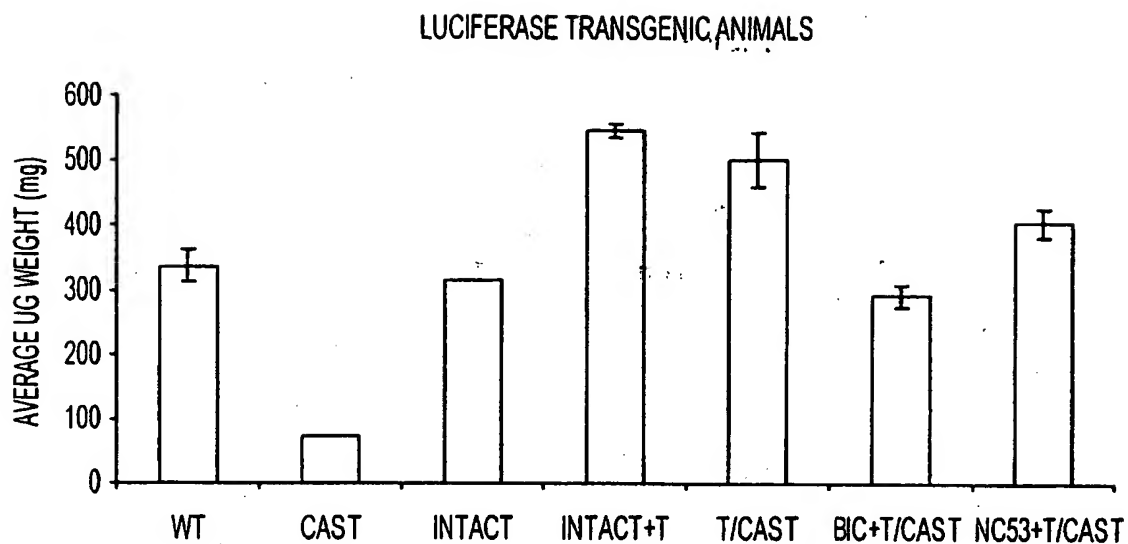


FIG. 22A

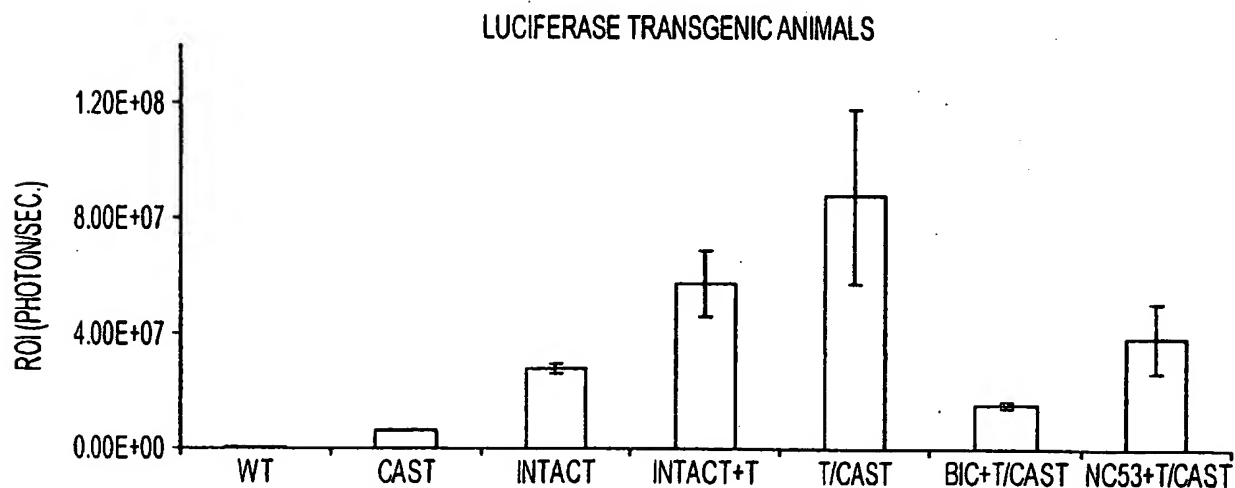


FIG. 22B

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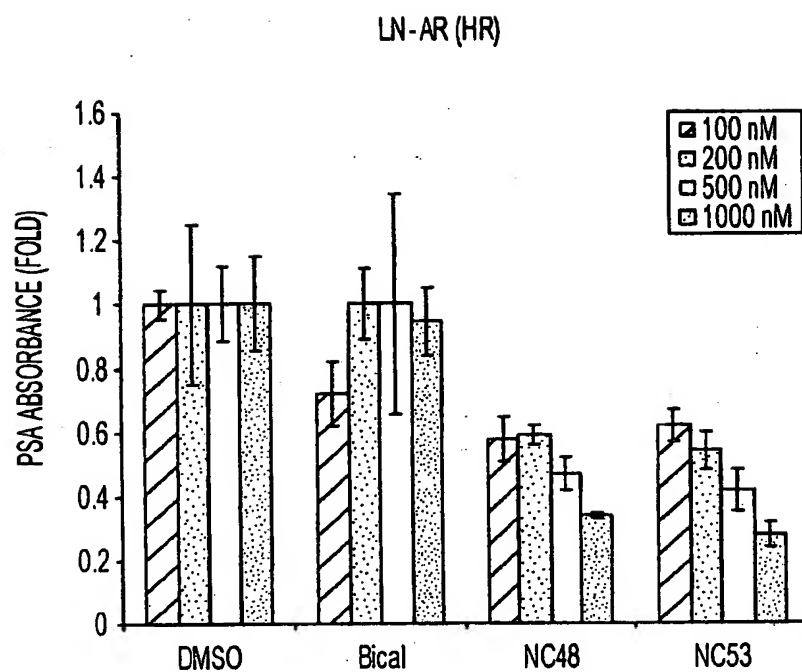


FIG. 23

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CHARACTERISTICS OF Bicalutimide, NC7, NC48 AND NC53

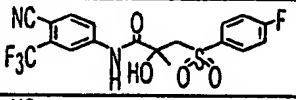
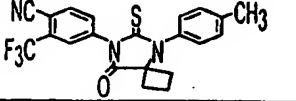
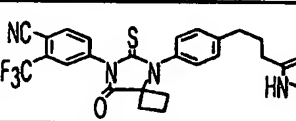
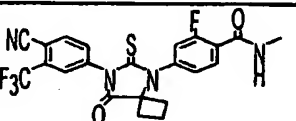
| NAME | STRUCTURE | IC ₅₀ [nM] | LogP | C _{SS} , 10mpk [μM] | C _{SS} , 25mpk [μM] | C _{SS} , 50mpk [μM] |
|------|---|--------------------------|------|---------------------------------|---------------------------------|---------------------------------|
| Bic. |  | 1000 | 2.91 | 10.0 | 11.4 | 11.9 |
| NC7 |  | 124 | 4.20 | NA | NA | NA |
| NC48 |  | 92 | 3.44 | 0.39 | 0.43 | 0.40 |
| NC53 |  | 122 | 3.20 | 9.9 | 10.7 | 10.2 |

FIG. 24A

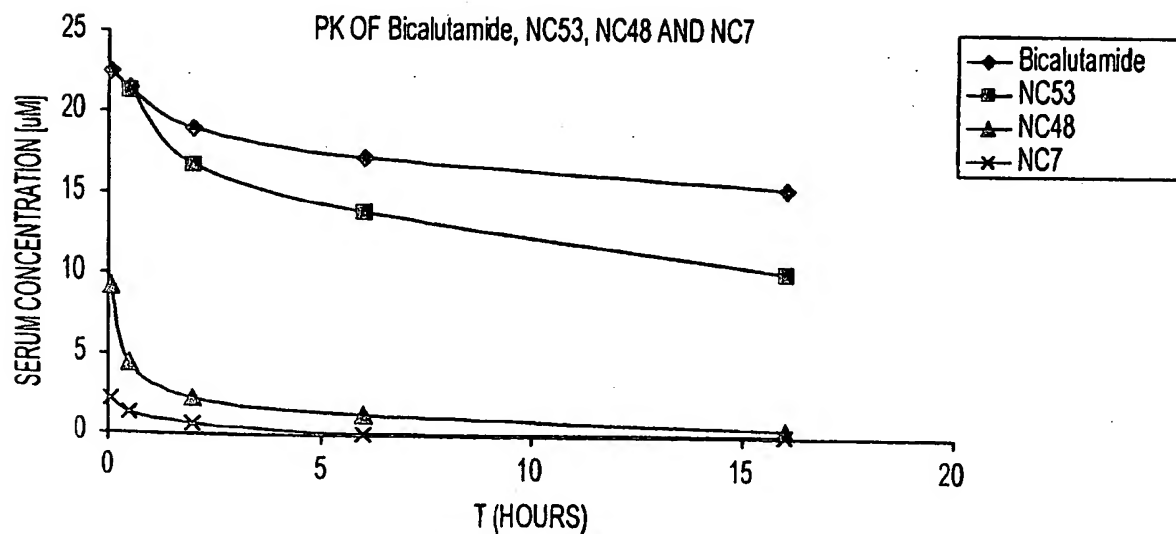


FIG.24B

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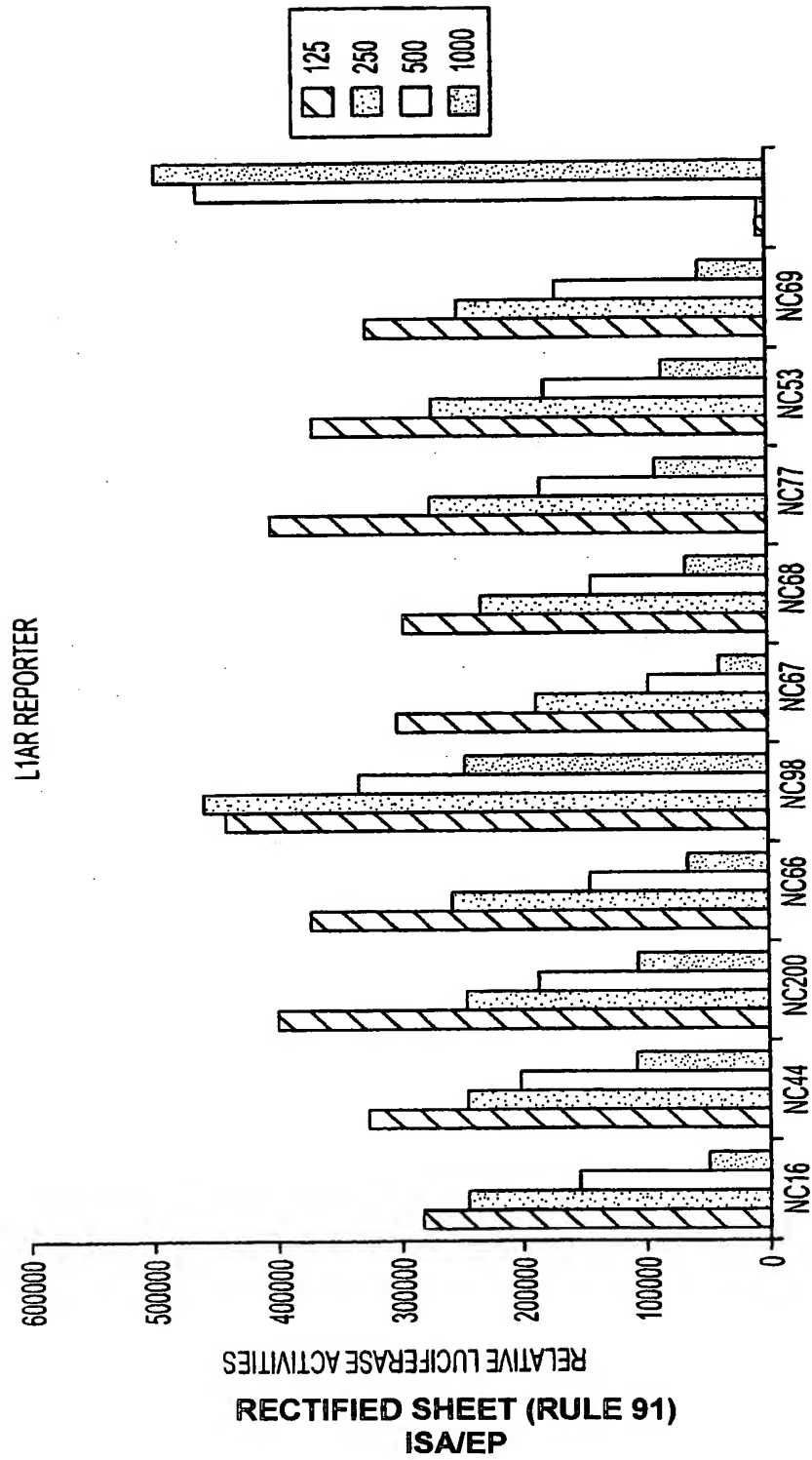


FIG. 25